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Environmental and Intrinsic Control of Filtering and Feeding Rates in Arctic *Daphnia*

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CHISHOLM, S. W., R. G. STROSS, AND P. A. NOBBS. 1975. Environmental and intrinsic control of filtering and feeding rates in arctic *Daphnia*. J. Fish. Res. Board Can. 32: 219–226.

Filtering and feeding rates of *Daphnia middendorffiana* at Point Barrow, Alaska were measured as a function of temperature, food density, animal length, and time of day.

The functional relationship between feeding rate and food density followed the hyperbolic pattern typical for filter feeders. *Daphnia middendorffiana* was found to have comparatively high filtering capabilities at its temperature optimum (around 11 C), with filtering rate increasing with length according to the equation $F = .458L^{3.17}$. The exponent of this equation was found to be a function of temperature, and the coefficient a function of food density.

A bimodal diel rhythm in feeding rate was found in this species in experiments where the animals were held under constant conditions. Feeding rates were maximum at 1400 and 2400, the time of day when the temperature cycle in the ponds passes through the mean daily temperature, which is optimum for feeding in *D. middendorffiana*. Thus the timing of the rhythm may serve to maximize the daily food gathering ability of this species.

CHISHOLM, S. W., R. G. STROSS, AND P. A. NOBBS. 1975. Environmental and intrinsic control of filtering and feeding rates in arctic *Daphnia*. J. Fish. Res. Board Can. 32: 219–226.

Nous avons mesuré les taux de filtration et d'alimentation de *Daphnia middendorffiana* à Point Barrow, Alaska, en fonction de la température, densité de la nourriture, longueur de l'animal et heure du jour.

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La relation fonctionnelle entre le taux d'alimentation et la densité de la nourriture a la forme hyperbolique typique des filtreurs. Nous avons constaté chez *D. middendorffiana* une capacité de filtration relativement élevée à son optimum de température (aux environs de 11 C), le taux de filtration augmentant en fonction de la longueur selon l'équation $F = .458L^{3.17}$. L'exposant de cette équation est fonction de la température, et le coefficient fonction de la densité de la nourriture.

Nous avons observé un rythme nycthéméral bimodal dans le taux d'alimentation de cette espèce, dans des expériences au cours desquelles les animaux ont été maintenus dans des conditions uniformes. Les taux d'alimentation sont à leur maximum à 1400 et 2400 heures, heures correspondant au point de rencontre du cycle des températures dans les étangs avec la moyenne journalière de température, qui est optimale pour l'alimentation de *D. middendorffiana*. Le chronométrage du rythme peut donc servir à maximiser l'aptitude de cette espèce à capturer sa nourriture quotidienne.

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FILTERING rates of zooplankton are a function of a number of intrinsic and extrinsic variables. Rigler (1961) has shown that filtering rates in *Daphnia magna* Straus are invariant with increasing food concentration until a critical concentration is reached. Food concentrations greater than this cause a decline in the filtering rate. The result in terms of particle ingestion (feeding rate), is a hyperbola which saturates at the critical concentration (Rigler 1961; McMahon and Rigler 1963, 1965; Burns and Rigler 1967; McMahon 1968). Filtering rates of *Daphnia* are also affected by food species and the size of food particles (McMahon and Rigler 1965; Burns 1969), temperature (McMahon 1965; Burns and Rigler 1967; Schindler 1968; Burns 1969; and Kibby 1971), and animal length (Ryther 1954; Richman 1958; Burns 1969; Burns and Rigler 1967).

The purpose of this investigation was to measure the parameters that determine filtering and feeding rates in arctic *Daphnia* and to explore the intrinsic and environmental variables that influence these parameters. It is reasonable to expect that the basic functional relationships and their determinants for an arctic species would resemble those for its temperate counterpart. The real focus of this investigation, therefore, was to detect specific characteristics of the functional relationships that could reflect unique adaptive properties of arctic *Daphnia*.

Daphnia middendorffiana Fischer, the dominant filter feeder in depression ponds of the arctic plain (mean depth 0.2 m), was chosen as the experimental animal. The development of this species throughout the short arctic growing season is nearly synchronous, with most of the animals hatching from resting eggs within a few days in the spring and having a single brood of young in mid-July. This type of population synchrony offers a unique opportunity for physiological studies on organisms obtained from the field, in that it provides assurance that all experimental animals are of the same age and have similar past histories.

Methods

Filtering rates were determined using the short-term feeding method of Nauwerk (1959), where animals are fed radioactively labelled food over a period of time short enough to eliminate losses from defecation and excretion. A filtering rate determined in this way is defined as the volume of ambient medium swept free of cells by one animal in a given time interval (assuming that the labelled cells are not selectively gathered). Accordingly, feeding rate is the number of cells ingested by that animal per unit time. Either of the two rates is easily calculated from the other if the food density is known. Results of individual experiments presented here are expressed in terms of that rate which provided easy comparisons with published data for other species of *Daphnia*.

Radioactively labelled (^{14}C) *Chlamydomonas reinhardti* Dangeard, cultured in nutrient-enriched, autoclaved pond water, was added to phytoplankton from the source ponds to act as the labelled food source. *Chlamydomonas* cells were labelled by adding 120 μCi $NaH^{14}CO_3$ to 50 ml of culture over a period of 3 days. Before each experiment, the labelled cells were centrifuged, the resulting pellet was resuspended in filtered (64-μ pore size) pond water, and cells were diluted to desired concentrations. Unlabelled cells were processed similarly and used to acclimate the animals to experimental food densities. The concentration of labelled *Chlamydomonas* added to the natural assemblage enriched the natural phytoplankton density by at least 50%. Cells in the feeding suspension were counted with a Coulter Model B electronic particle counter (Coulter Electronics, Hialeah, Fla.).

Before each experiment, *Daphnia* were collected from ponds on the US-IBP study area Site 7 near Barrow, Alaska (71°N, 156°E), concentrated through a 200-μ pore size net, and conditioned for at least 1 h at the temperature that would be used in the

experiment. Temperatures in the range of those found in the source ponds (about 5, 10, and 15 C) were used. Unless otherwise noted, all experiments were run between 1200 and 1400 (ADT). The protocol for the diel experiments deviated from this procedure to some extent and is described below.

A multiple chambered feeding "gang" was developed to allow six simultaneous feeding measurements (Fig. 1). A 30-ml suspension of labelled food was placed in each side arm and groups of 5–15 *Daphnia* (depending on their size) were placed in the main tubes in 30 ml of unlabelled food at the same concentration as that in the side arms. After the *Daphnia* had fed on unlabelled food for 15 min, all six of the chambers were tipped in unison, allowing the labelled food from the side arm to flow into the main chamber. The *Daphnia* were allowed to feed for 5–8 min, at which time the contents of the chambers were simultaneously emptied into Buchner funnels (fitted with 200-μ pore size netting) to separate the *Daphnia* from the food source. The liquid was then withdrawn from the funnels rapidly with gentle suction. Groups of animals were gently removed from the netting with dissecting forceps and placed in a scintillation vial containing 0.5 ml Protosol (New England Nuclear). Two 0.5 ml aliquots of the filtrate, containing the labelled food source, were placed in scintillation vials for counting radioactive food available. The remainder of the food solution was set aside for counting cell density with a Counter Counter.

Sample preparation of the *Daphnia* before liquid scintillation counting included a 24-h incubation in Protosol at room temperature, followed by maceration with a tissue homogenizer, and suspension in 10 ml of scintillation cocktail.

Filtering rate was calculated with the equation:

$$F = Dx/Ant \qquad (1)$$

where D = counts/minute in the zooplankton, A = counts/minute in the algae, x = milliliters algae counted, n = number of *Daphnia* counted, and t = incubation time in hours.

From this, feeding rate can be calculated with the equation:

$$f = FC \qquad (2)$$

where F = filtering rate in milliliters/animal per hour, and C = concentration of food particles in cells/milliliter.

Results

Feeding and Filtering Rate as a Function of Food Density

Filtering rates of the *Daphnia middendorffiana* population were measured as a function of food concentration at 11 C several times over the summer of 1972. Feeding rates were calculated from these data. On all occasions feeding rate was found to be a hyperbolic function of food density and filtering rate was characteristically inhibited at the higher densities. Representative results showing the functional relationships for one day (July 26) are shown in Fig. 2. The saturated feeding rate (f_{max}) of adult *Daphnia* decreased considerably as the summer progressed. The highest f_{max}, 265,000 ± 76,000 cells/animal per hour, occurred on July 5 when the animals were 2.3 mm long. On July 8 the same sized animals had an f_{max} of 159,000 ± 11,000. On July 14, 21, and 26 the animals were 2.6 mm long and the f_{max} values were 112,000 ± 14,000, 98,000 ± 14,000, and 37,000 ± 6,000, respectively. A decline in the feeding rates of aging zooplankton has also been observed by Ryther (1954) in *Daphnia* and by Reeve (1963) in *Artemia*. On all

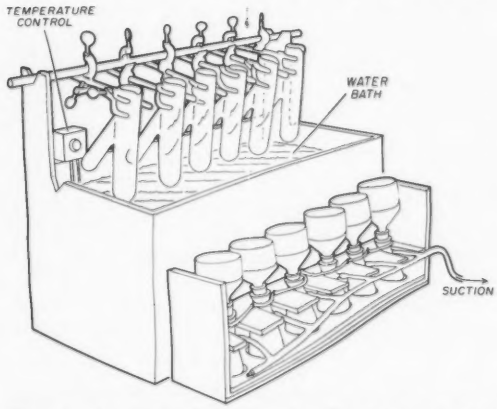

FIG. 1. Apparatus for measuring filtering rates. See text for details of operation.

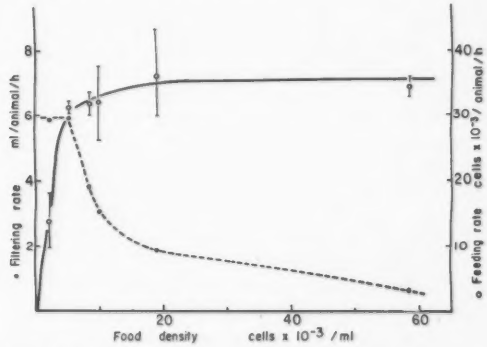

FIG. 2. Filtering rate (– – – –) and feeding rate (————) of *Daphnia middendorffiana* as a function of food density. The measurements were done at 11 C on animals 2.6 mm long.

but one occasion (July 8) f_{max} was reached at a cell density of less than 26,000 cells/ml.

Filtering Rate as a Function of Temperature

Maximum filtering rate was found near 12 C for all size-classes (age-groups) of *D. middendorffiana* studied (Fig. 3). This temperature is considerably lower than the maximum temperatures the species encountered during the summer, as water temperatures sometimes reached 18 C in the late afternoon of July 1972 (Stanley 1973).

Composite Effects of Animal Length, Temperature, and Food Density on Filtering Rate

Filtering rates of the *D. middendorffiana* population were measured periodically throughout the summer during which time the animals grew from 1.3 mm to 2.6 mm. Measurements were made at two temperatures and three food densities. Filtering rate was regressed on animal length for six combinations of temperature and food density using the equation:

$$\log F = b + \alpha \log L \qquad (3)$$

where F = filtering rate in milliliters/animal per hour, L = animal length in millimeters, α = a constant, and b = a constant, the value of which is dependent on the dimensions of L and F. Equation (3) is a log–log transformation of the power function:

$$F = bL^{\alpha} \qquad (4)$$

and is used to facilitate regression calculations.

In all cases, filtering rate was found to increase with animal length, and the data were adequately described by equation 4 (e.g. Fig. 4). The magnitude of the increase, dictated by the values of α and b in equation 4, was a function of the temperature and food density at which the filtering rates were measured (Table 1). As expected, filtering rates were inversely related to food density and directly related to temperature. The specific effects of these two variables on the exponent and coefficient of equation 4, however, indicates that temperature and food density influence filtering rate in distinctly different ways (Table 1). The coefficient, b, decreases with increasing food density but is unaffected by temperature. The value of the exponent, α, however, is not significantly influenced by food density (comparison of slopes, Sokal and Rohlf 1969) but increases with increasing temperatures. The increase in the exponent is statistically significant ($P = 0.05$) only at the lowest food concentration, although the increase is still evident at the other two concentrations.

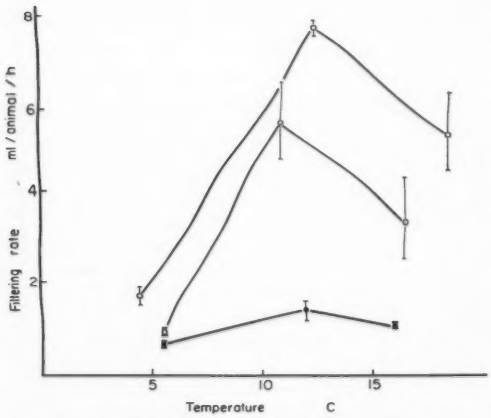

FIG. 3. Filtering rate of *D. middendorffiana* as a function of temperature for three size-classes: 1.3 mm (●), 2.3 mm (○), and 2.6 mm (□).

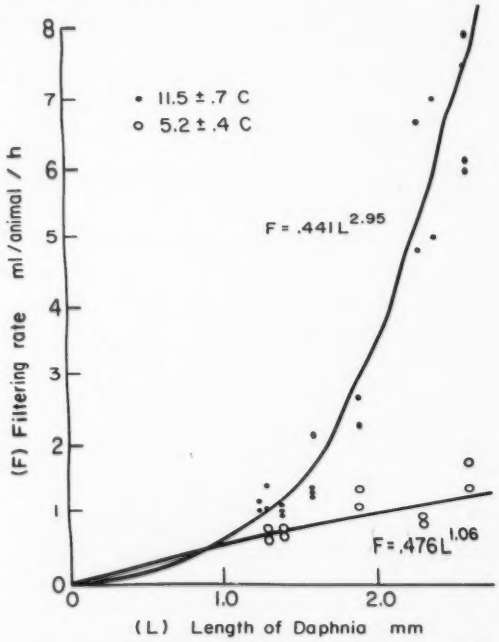

FIG. 4. Temperature dependence of filtering rate in *D. middendorffiana* as a function of animal lengths. Data points and regressions are shown.

TABLE 1. Equations describing filtering rate (F) of *Daphnia middendorffiana* as a function of animal length (L) at six combinations of temperature and food density.

Temp (C)	Food density (cells/ml)		
	8,317±3,266	25,247±5,701	82,957±24,233
5.2±0.4	$F = 0.476\ L^{1.06}$	$F = 0.176\ L^{2.04}$	$F = 0.083\ L^{1.86}$
11.5±0.7	$F = 0.441\ L^{2.95}$	$F = 0.173\ L^{3.41}$	$F = 0.083\ L^{3.15}$

DAILY RHYTHMS IN FEEDING RATE

Daily oscillations in feeding activity were observed in *D. middendorffiana* during two long-term experiments which were run 10 days apart late in the summer. The protocol for each experiment was the same. *Daphnia* were collected 14 h before the onset of the experiment (2400) and stored in two 20-liter carboys in dim light at 5 C, which is near the daily minimum temperature in the source ponds. The animals were maintained in this way to eliminate oscillatory stimuli whose effects might carry over to the experimental period. The potential danger that extended preconditioning at 5 C could cause acclimation phenomena similar to those described by Kibby (1971) is recognized, but evidence that this occurred was not apparent in the data. Feeding rates were measured on *Daphnia* taken from the carboys 6 times over a 24-h period, beginning at 1400. Food densities used were 6100 ± 900 cells/ml in experiment 1 and 4250 ± 50 cells/ml in experiment 2. Measurements were made at 5 and 10 C in the first experiment and 5 and 12 C in the second; the second temperature was always measured within 1.5 h of the first, and the animals were preconditioned for 1 h at the experimental temperature.

In both experiments the daily oscillation in feeding rate was bimodal (Fig. 5A). Peaks in activity consistently fell at 2400 and 1400, with feeding rates at the maxima approximately double those at the minima.

The rhythm in feeding activity at 10 and 12 C was accompanied by a rhythm in temperature sensitivity (Fig. 5B). Temperature sensitivity, for our purposes, is defined as the change in feeding rate per unit change in temperature. It reflects the capacity of the organisms to respond to increases in temperature. The two intervals when the organisms were most sensitive to temperature (1400 and 2400) correspond with the intervals of maximum feeding rates found at the higher temperatures (Fig. 5A). In both experiments, negative temperature sensitivity, reflecting slight inhibition by the higher temperatures, was found at 1800, a time corresponding to near maximum water temperatures for clear summer days (Fig. 5C).

Discussion

Daphnia middendorffiana has the largest filtering capabilities of any species of *Daphnia* investigated. A simple comparison of species can be made by examining the coefficients and exponents that relate filtration rate to animal length (Table 2). The large values of the coefficient and exponent for the arctic species (at 11 C) indicate that, when compared with an individual of the same length belonging to another species, *D. middendorffiana* will always have the higher filtering rate. The large filtering power, combined with the large size of this species (maximum 3.2 mm), makes it a very effective feeder. The high filtering capabilities of *D. middendorffiana* may reflect an adaptation to the low primary productivity found in the ponds it inhabits (Alexander et al. 1972). Also reflecting an adaptation to a depauperate food supply is the low incipient saturating food concentration for this species. The feeding rate of *D. middendorffiana* saturates at food concentrations less than 26,000 cells/ml, which is a considerably smaller value than that found for temperate species of *Daphnia* (Rigler 1961; McMahon and Rigler 1965; Burns and Rigler 1967).

The temperature optimum for feeding in *D. middendorffiana* is, as expected, lower than that reported for temperate species (Burns and Rigler 1967; Burns 1969). Kibby (1971) has shown that within a range of 12 and 20 C, the temperature optimum for filtering rate in *Daphnia* can be entirely a function of the temperature at which the animals were grown. The low temperature optimum in *D. middendorffiana*, therefore, could reflect acclimation rather than adaptation.

The effect of temperature on the relationship between length and filtering rate is interesting in its aparent specificity for the exponent of equation 4 (Table 1). Burns (1969) has also observed this

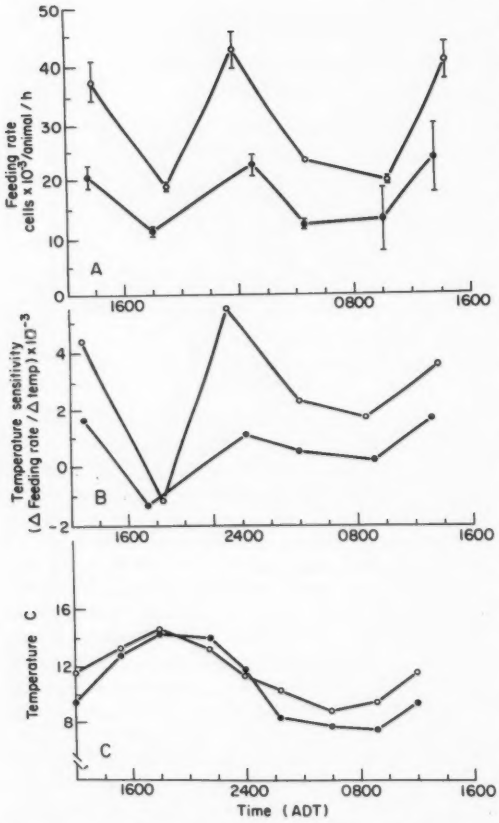

FIG. 5. A, Feeding rate of *D. middendorffiana* as a function of time of day. Experiments were run on July 21–22 (●) and August 1–2 (○) at temperatures of 10.2 ± 0.6 C, and 11.7 ± 0.8 C, respectively. B, Temperature sensitivity, defined as the change in feeding rate per unit change in temperature, as a function of time of day. The dates of the experiments are as given in Part A. The temperatures in the first experiment were 5.1 ± 0.2 C and 10.2 ± 0.6 C and in the second, 4.9 ± 0.5 C and 11.7 ± 0.8 C. Points are plotted in the middle of the 1.5-h interval over which the measurements were made. C, Mean daily temperature cycles for the 4 days preceding each experiment shown in Parts A and B. Data from Stanley (1973).

phenomenon in four other species, although the reduction in the value of the exponent in her equations was not as extreme as that found for *D. middendorffiana* (Table 2). Although the decrease in the exponent with decreasing temperature is quite convincing from the regression analysis, it is difficult to justify this relationship with a biological mechanism. The geometry of the filtering process dictates that filtering rate should increase as the cube of the length of the animal (Egloff and Palmer 1971) and it is difficult to explain how a lowering of temperature could alter this geometry. If the cubic relationship is mathematically forced at the lower temperatures, the function generated generally fits the data points, but consistently underestimates the filtering ability of animals shorter than 2 mm and overestimates the ability of those that are longer. In dealing with the length-filtering rate relationship, therefore, one is faced with a fundamental dilemma: Does one accept the results of the regression analysis and conclude that the geometrical interpretation of the system is oversimplified, or does one force the cubic relationship, and try to determine what factors are causing the observed data to deviate? This question cannot be resolved with our present knowledge of the filtering mechanism.

It is not surprising to find daily rhythms in feeding rate and thermal sensitivity in arctic *Daphnia*. Daily rhythms are well documented in the genus and include bimodal field rhythms in vertical migration (McNaught and Hasler 1964) which have been shown to have an endogenous basis (Enright and Hamner 1967; Harris 1963) and circadian rhythms in phototaxis (Ringleberg and Servaas 1971). The fact that arctic species of *Daphnia* are capable of photoperiodic time measurement (Stross 1969) indicates that activities in these species can be controlled by a circadian clock. In our experiments, the time studies of filtering rates were run under constant conditions with individuals that had been held under constant conditions for 14 h before the experiments. We believe, therefore, that the rhythm is intrinsic at least and suppose it to have a self-sustaining driver, although this was not tested.

The bimodal nature of the feeding and temperature sensitivity rhythms in *D. middendorffiana* corresponds with activity patterns in some temperate latitude *Daphnia*, copepods and insects, and in arctic algae. Spindler (1971a, b) has shown double peaks for egg laying and molting in *Cyclops vicinus* with 12-h intervals between the midday and night peaks. Rensing et al. (1965) show bimodal oscillations in the intracellular activity of neurosecretory and endocrine gland cells of *Drosophila melanogaster*, and Müller-Haeckel (1970) shows that the colonization activity of diatoms in an arctic brook may be bimodal in some species.

It could be proposed that the bimodal nature of the rhythms observed in *D. middendorffiana*

TABLE 2. Comparison of filtering rate and animal length relationships for seven species of *Daphnia*.

Species	Temp	Range of lengths	Length function	References
D. middendorffiana	5 C	1.3–2.6 mm	[a]$F = .458L^{1.65}$	Present study
"	11 C	"	[a]$F = .458L^{3.17}$	Present study
D. rosea	20 C	0.64–1.6 mm	$F = .310L^{3.02}$	Burns and Rigler 1967
D. pulex *D. schødleri* *D. magna* *D. galeata*	15 C 20 C	0.75–4.0 mm "	$F = .153L^{2.16}$ $F = .208L^{2.80}$	Burns 1969 "
D. magna	20 C	1.25–3.54 mm	$F = .280L^{2.20}$	McMahon 1965

[a]Exponents (within temperatures) and coefficients (at lowest food density) were averaged from Table 1.

results from a splitting of the circadian rhythm by the continuous light of the arctic summer (see Hoffmann 1971). However, Swade and Pittendrigh (1967) have shown that activity rhythms in vertebrates can be entrained (with single peaks of activity each day) by illumination cycles with amplitudes comparable to those encountered at Point Barrow during the summer solstice. In contrast, some invertebrates are reported to be desynchronized by the long photoperiods of high latitudes (Müller 1970).

An alternative explanation for the bimodality of the feeding pattern in *D. middendorffiana* is that there are two temporally distinct populations of this species, one with a day activity peak at 1400 and one with a night peak at 2400. Indeed, the larval stages of certain insect species have been shown to be night active, while the adult stage is active during the day (Mendl and Müller 1970). The splitting of the *Daphnia* into two subpopulations could act as a mechanism for reducing intraspecific competition by stratifying the intervals of maximum feeding activity over 24 h. Unfortunately, to establish firmly that two subpopulations exist in the arctic ponds would require monitoring a single *Daphnia* over the entire cycle, which is not feasible with our techniques.

Whatever the mechanistic explanation for the bimodal pattern, it is potentially significant that the activity peaks in *Daphnia* feeding activity at Barrow correspond with the times each day that the temperature in the ponds passes through the thermal optimum for this species. That is, water temperatures in the ponds may oscillate between 5 and 15 C on a clear day with temperatures passing through the 11 C point when the *Daphnia* appear to be maximally active. Since the daily maximum, and usually the daily minimum, temperature suppresses filtering rate, it could be argued that maximizing activity at the optimum temperature is advantageous. This is apparently what arctic *Daphnia* do.

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Oxidative Deterioration of Canned Lake Whitefish (*Coregonus clupeaformis*) Treated with Antioxidants

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BIGGAR, C. A., N. A. M. ESKIN, M. VAISEY, AND B. JOHNSTON. 1975. Oxidative deterioration of canned lake whitefish (*Coregonous clupeaformis*) treated with antioxidants. J. Fish. Res. Board Can. 32: 227–232.

Lake whitefish (*Coregonus clupeaformis*) was canned with one of two antioxidant mixtures, AX1 (20% butylated hydroxyanisole, 20% butylated hydroxytoluene) or AX2 (10% butylated hydroxyanisole, 10% butylated hydroxytoluene, 6% citric acid, and 6% propyl gallate), and compared to untreated control samples. The flavor and quality of the fish were examined at intervals during 4 days of open refrigerated storage using sensory, chemical, and physical methods. Both thiobarbituric acid (TBA) values and the results of paired flavor comparisons by an eight-member trained sensory panel demonstrated that the greatest degree of oxidative deterioration was always associated with the untreated fish, while AX1-treated fish exhibited the least amount of "off-flavor" over the storage period. The relative degree of "off-flavor" as evaluated by the panelists tended to increase on storage; TBA values showed a linear relationship with time. Tristimulus readings using the Hunter Colorimeter showed a significant difference between the control sample and the two antioxidant-treated samples with respect to the pink color of freshly opened canned whitefish.

BIGGAR, C. A., N. A. M. ESKIN, M. VAISEY, AND B. JOHNSTON. 1975. Oxidative deterioration of canned whitefish (*Coregonus clupeaformis*) treated with antioxidants. J. Fish. Res. Board Can. 32: 227–232.

Des grands corégones (*Coregonus clupeaformis*) ont été mis en conserve avec l'un ou l'autre de deux mélanges antioxydants, AX1 (hydroxyanisol butylé 20%, hydroxytoluène 20%) ou AX2 (hydroxyanisol butylé 10%, hydroxytoluène butylé 10%, acide citrique 6% et gallate de propyle 6%) et ont été comparés avec des échantillons témoins non traités. A l'aide de méthodes sensorielles, chimiques et physiques, nous avons étudié la saveur et la qualité du poisson périodiquement, durant 4 jours d'entreposage réfrigéré ouvert. Les valeurs d'acide thiobarbiturique (ATB) et les résultats de comparaisons de saveur en paires par un groupe de dégustation de huit professionnels démontrent tous deux que la plus forte détérioration par oxydation est toujours associée au poisson non traité, alors que le poisson traité à l'AX1 est celui qui subit le moins de changement de saveur durant la période d'entreposage. Le degré relatif de changement de saveur, tel qu'évalué par le groupe de dégustation, a tendance à augmenter avec la durée d'entreposage; les valeurs d'ATB démontrent une relation linéaire en fonction du temps. Les lectures de tristimulus au colorimètre de Hunter indiquent une différence significative, sous le rapport de la coloration rose du grand corégone d'une boîte de conserve qui vient d'être ouverte, entre l'échantillon témoin et les deux échantillons traités à l'antioxydant.

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LAKE whitefish (*Coregonus clupeaformis*) is an important commercial species of freshwater fish in Canada. Since whitefish is a highly perishable product, attempts to extend its storage life have been reported, including freezing (Osterhaug 1956; Awad et al. 1969), irradiation (Ostovar et al. 1967), and smoking (Slusar and Vaisey 1970). Canning the fish, however, appears to be the best method of extending the storage life since it excludes air, inactivates enzymes, and destroys spoilage organisms.

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The canned whitefish product has a characteristic flavor which has been described as "chickeny." This quality, however, appears to be unstable and changes within minutes of opening the can to a "fishy" taste which is thought to be associated with fat oxidation (Shaykewich 1971). It has also been noted that the color of the canned whitefish changed from pink to white after opening. In a report to the Manitoba Department of Industry and Commerce, Arthur L. Little, Inc. reported that canned whitefish could compete cost-wise with canned salmon and canned tuna (Anon. 1965). Since canned fish is an acceptable product, the potential market for this form of whitefish appears promising, as long as the product is of sufficiently high quality.

Earlier studies on canned whitefish were directed to the correction of too soft texture (Shaykewich et al. 1972). A recent study by Sweet (1973) suggested that a proper distribution of phenolic antioxidants might substantially increase the shelf life of frozen freshwater fish. The purpose of the present study was to extend the time that the flavor of canned whitefish remains highly acceptable after opening through the addition of antioxidants prior to canning.

Materials and Methods

Lake whitefish (*Coregonus clupeaformis*) was canned with and without antioxidants and evaluated after open refrigerated storage using organoleptic, chemical, and physical measurements.

The fish used in this study were caught by gillnet in May 1971 in William Lake, Manitoba, and processed within 48 h of landing (Schwartz 1973).

There were three treatments applied to the whitefish, in which one of the following was added prior to canning: 1) a mixture of 20% butylated hydroxyanisole (BHA), 20% butylated hydroxytoluene (BHT), and 60% vegetable oil carrier[2] (AX1); 2) a mixture of 10% BHA, 10% BHT, 6% citric acid, 6% propyl gallate (PG), propylene glycol, monoglyceride citrate, and a vegetable oil carrier[2] (AX2); 3) no added antioxidant.

The total amount of antioxidant accounted for 0.146 and 0.117% of the fat or oil content of the fish for AX1 and AX2, respectively. Fat content was assumed to be a constant 2%, although in whitefish it is known to vary from 1.7 to 3.9% in fish from Cedar Lake or Lac la Rouge to 16.3% in fish caught in Lake Huron (Bligh 1971). The antioxidants were added to empty gelatin capsules[3] by a Winetraub dropper, using gravity alone.

To reduce the variability as much as possible, the treatment with no antioxidant received an empty capsule. In addition to the gelatin capsule with or without antioxidants, a salt pellet (2 g) was added to each 100-g fish and the cans were vacuum sealed and sterilized at 115 C for 1 h. The cans were allowed to cool, and then labelled and stored at room temperature for a minimum of 30 days to obtain good flavor and aroma distribution (Anon. 1966). After this aging period, the cans were refrigerated in household-style refrigerators prior to examination.

Sensory evaluation — An eight-member panel of graduate students and staff members of the Department of Foods and Nutrition were trained to recognize off-flavor in canned whitefish. The trained panel evaluated opened cans of whitefish which had been subjected to 0, 1, 2, 3, and 4 days of aging in a household-style refrigerator.

Using a paired comparison method with a degree of difference scale (Table 1), every panelist compared each of the two antioxidant treatments to the untreated fish at each of the five time intervals, in sequence. Accordingly there were five sessions required for the experiment with the panelists each examining two pairs (zero, AX1; zero, AX2) at a session. The order of sample presentation within a pair was arranged so that one-half the judges received the untreated control sample first and the other half tasted an antioxidant-treated sample first. The order of pair presentation was randomized. Samples were examined in individual booths in a sensory testing room where red lighting was used to mask any color differences. The sensory experiment was repeated 3 times. While the panel always consisted of eight judges, some substitutions in membership became necessary during the trial for reasons unrelated to performance. A total of 15 people were involved as judges in the total exercise.

All the cans of fish for one replication were opened at the beginning of the storage study. The fish was fork-flaked to uniformity. Duplicate bowls of six to eight tins of fish were kept for each treatment. The individual bowls were covered with plastic film[4] and the samples aged in a home-style refrigerator at 10 C. Samples were taken from each of the two bowls on each test day to give duplicates for the TBA test and four samples for the sensory evaluation. Sensory samples from both bowls were assigned to the eight panelists at random.

Chemical and physical evaluations — Thiobarbituric acid (TBA) values were determined according to the method described by Vyncke (1970). Samples from the combined contents of six to eight drained tins of whitefish were set up in duplicate for each treatment. TBA values were measured initially and following 1, 2, 3, and 4 days of storage in a household-style refrigerator. TBA values were also determined on fish samples to which 0.1 g EDTA (ethylenediaminetetra-

[2]Griffiths Laboratories.

[3]Lilly empty gelatin capsules No. 3. Eli Lilly and Company, Indianapolis, Ind. 46206, USA.

[4]Saran Wrap, Dow Chemicals Ltd., Montreal, Que.

TABLE 1. Questionnaire used by sensory panelists.

Comparison of flavors in canned whitefish
You are receiving two samples to compare for "off-flavor." 1. Taste the samples in the order listed below. 2. Circle the code number of the one which has more "off-flavor." 3. Indicate the degree of difference in off-flavor between the two samples. SAMPLE CODES ——— ———
AMOUNT OF DIFFERENCE Indicate the degree of difference between the two samples by marking the appropriate phrase on this scale.
Very slight · Slight · Moderate · Much · Very much · Extreme

acetic acid) and 0.1 g propyl gallate (PG) had been added prior to homogenizing. This experiment was performed twice.

Tristimulus readings were taken on the canned whitefish samples using the Hunterlab D25 Color Difference Meter. Of the three values obtained from this instrument (L, a, and b) the "a" values appeared the most pertinent for canned whitefish because of its definite pink or "shrimpy" color when first opened. Hunter "a" values indicate a range in colors from red (+) to green (–). Three individual cans of fish representing each of the three antioxidant treatments (zero, AX1, AX2) were fork-flaked in separate bowls. Duplicate samples were examined initially and after 6 h, 1, 2, 3, and 4 days. This test was not replicated.

Statistical analyses — For the sensory measurements, total scores (β values) measuring the degree of off-flavor for the control and each antioxidant treatment at each day of storage were derived following the procedure of Scheffé (1952). These total scores were then analyzed by multiple regression using a model based on Scheffé's analysis but modified to permit a comparison of the effects of both antioxidants in relationship to each other as well as to the control, even though AX1 and AX2 were not compared directly by the panelists (Johnston and Biggar 1974). An extension of this model was necessary to permit estimation of the time effect over days of storage.

Analyses of variance were performed on the TBA values and the tristimulus readings with data arranged as complex split plot designs (Snedecor and Cochran 1967) with duplicate error isolated from the other sources of variation. In the TBA analysis the whole plot sources were antioxidants, chelating agents, their interaction, replicates, and error; the split plot sources were days, the interaction of days with whole plot treatments, split plot error, and replicate error. Chelating agents and replicates were not a part of the tristimulus experiment. Orthoganol contrasts were used to determine significant differences among antioxidant treatments and among days of storage.

Results and Discussion

As shown by sensory scores (β values), whitefish canned with antioxidants had better flavor over 4-day open refrigerated storage than fish with no chemical protection (Fig. 1). This observation was substantiated by the large F value for treatments ($F_{2,191} = 130.63$). Both antioxidant treatments had less flavor advantage over the control initially than on subsequent storage days, indicating more flavor deterioration in the control.

While the main effect for days showed no significant difference, a significant days by treatment

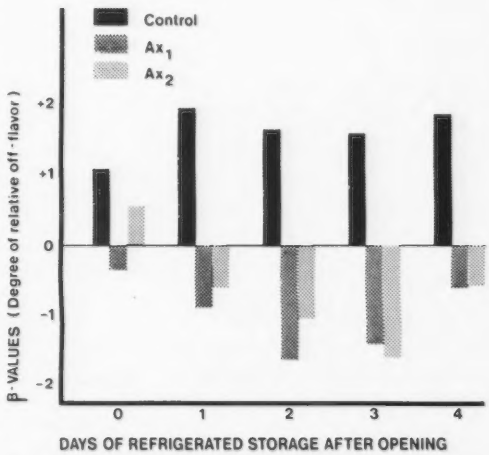

FIG. 1. Relative off-flavor of canned whitefish during refrigerated storage. A higher β-value indicates more off-flavor; each bar for AX1 and AX2 represents 22–24 judgments in comparison to the control; values for control derived (as in Schwartz 1973).

interaction ($F_{8,191} = 3.65$) pointed to a varying response of the treatments over time. The difference in flavor between the control and the antioxidant-treated samples became more marked during open storage, indicating more flavor deterioration in the control samples (Fig. 1). The relative scores (Fig. 1) show that the antioxidants used tended to vary in their flavor protective ability. AX1 gave fairly uniform flavor protection over time while AX2, which contained a lower proportion of the phenolic antioxidants, appeared less effective on the initial day and on the first 2 days of open refrigerated storage.

Chemical measurements supported and clarified the advantages of antioxidant use indicated by the sensory data. Unprotected whitefish experienced more rapid and extensive oxidative change during the 4 days of open refrigerated storage than the protected fish (Fig. 2). Sinnhuber and Yu (1958) and later, Zipser and Watts (1961), also demonstrated dramatic differences in TBA values between fish samples with and without added antioxidants.

In agreement with Vyncke's findings (1970), adding the chelating agents PG and EDTA to the fish prior to homogenizing significantly reduced TBA values ($F_{1,5} = 9.63$), suggesting that metals encourage oxidative changes in the fish during preparation for chemical testing. It is interesting that at Day 0, the control fish with chelating agents were as low in TBA value as the AX-treated fish, whereas the control homogenized without chelating agents showed appreciably more oxidation (Fig. 2). This resolves the observation that control fish had poorer flavor initially than AX-treated fish. Considering this effect of the chelating agents, it seems more likely that oxidative deterioration in the control occurred between opening the tin and tasting the fish, rather than during the canning process.

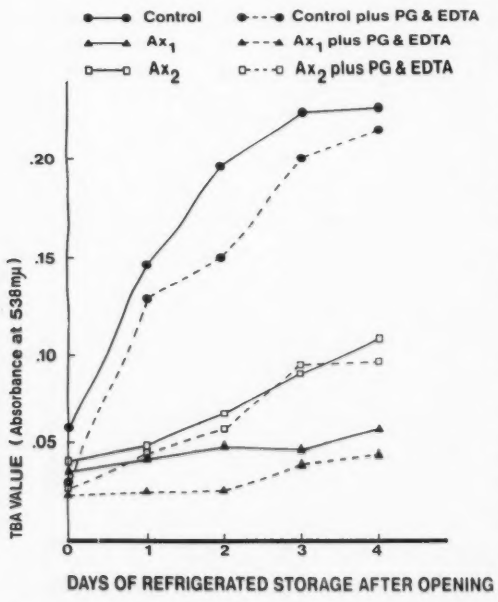

FIG. 2. Thiobarbituric acid values of canned whitefish during refrigerated storage.

While it is clear that oxidative changes increased over the storage period ($F_{4,24} = 148.03$), the significance of the AX by Days interaction ($F_{8,24} = 45.48$) pointed to a difference in the rate of changes among the respective treatments. Partitioning the variance of this interaction showed a strong linear effect of time on the degree of oxidation ($F_{2,24} = 1490.00$). The linear effect suggests that for each day of refrigerated storage after canned whitefish is opened, the TBA values could be expected to increase by 0.005 units in the presence of AX1, 0.007 units in the presence of AX2, and 0.044 units in the absence of chemical protection. However, the equations involving the slopes for each treatment (Fig. 3) give only approximations of the storage effects because a quadratic effect was indicated for the control. The observed values (Fig. 3) show that the straight lines overstate the differences in oxidation between the control and the AX treatments at Day 0, but understate them at Day 3.

While both antioxidants afforded protection from oxidative change, AX1 was more effective than AX2 (Fig. 3; $F_{1,24} = 30.17$). This may not be altogether unexpected since AX1 is associated with more antioxidant than AX2. The compatability of this finding with the flavor advantage of AX1 over AX2-treated fish discussed earlier suggests that the TBA value is a reasonably good indicator of oxidative flavor deterioration in canned whitefish.

Color differences among the whitefish treatments examined also showed significant differences due to AX treatment ($F_{2,6} = 11.58$) and storage time ($F_{4,45} = 177.00$). Hunter "a" values decreased in all treatments as refrigerated storage of the opened tins was extended (Fig. 4). The linear relationship of pink color with time was significant ($F_{1,24} = 662.25$). As with the sensory and TBA data, AX1 consistently afforded better color protection than AX2.

These studies have shown a beneficial effect of phenolic antioxidants on the maintenance of op-

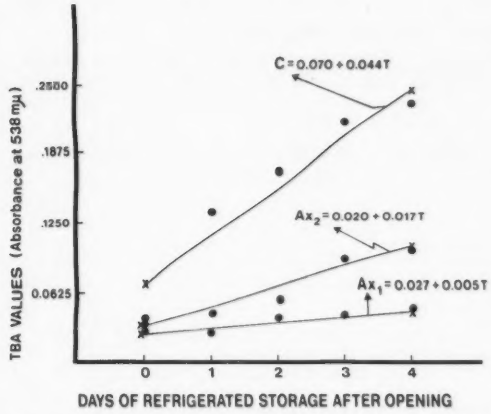

FIG. 3. The effect of refrigerated storage time on oxidative changes in canned whitefish.

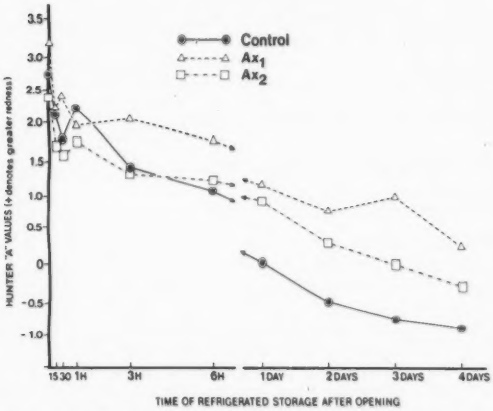

FIG. 4. Changes in redness (Hunter "a" Values) in canned whitefish during refrigerated storage. Values between 0 and 1 day were not used in the statistical analysis.

timal flavor and color of canned whitefish during refrigerated storage after the container has been opened. While a 4-day "leftover" storage period may be unlikely in many homes, evidence has been presented that detectable adverse oxidation occurs very soon after opening the can of fish. Ill effects of oxidation intermediates on nutritional quality are suspected (Labuza 1971; Osner and Johnson 1968). It seems worthwhile, then, that in the development of canned whitefish for maximum consumer acceptance, the use of phenolic antioxidants should be considered. The data from Fig. 3 were used to predict the TBA values of fish canned with the permitted level of phenolic antioxidant (0.02% of fat) on Day 1. With this time of open refrigerated storage the TBA value could be expected to be 0.118 in comparison to 0.135 for the untreated control. Further studies using these antioxidants within the levels permitted by the Food and Drug Regulations are necessary to ascertain their effectiveness under these conditions.

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Movements of Sockeye Salmon (*Oncorhynchus nerka*) in the Skeena River Estuary as Revealed by Ultrasonic Tracking

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Movements of adult sockeye salmon (*Oncorhynchus nerka*) entering the Skeena River were examined in 1969 and 1970 by ultrasonic tracking methods. Fifteen of 18 sockeye released in the lower river seemed to move passively in and out with flood and ebb streams. Two fish moved upstream independent of tides and one salmon swam against ebb and flood currents. Ground speeds in both years of operation were 1.6 km/h during rising and 2.1 km/h during falling tides, causing the fish to be transported downstream by about 3 km per tidal cycle. Three salmon released outside the river mouth in salt water also seemed to ride the tidal flows passively. Ground speeds during ebb (3.6 km/h) were again greater than during flood (2.0 km/h), indicating a net offshore movement. We conclude that these passive movements are not an artifact but that sockeye salmon normally slow down or pause upon reaching the "home river" and drift for a period in tidal currents in the estuary and river mouth before migrating upstream.

GROOT, C., K. SIMPSON, I. TODD, P. D. MURRAY, AND G. A. BUXTON. 1975. Movements of sockeye salmon (*Oncorhynchus nerka*) in the Skeena River estuary as revealed by ultrasonic tracking. J. Fish. Res. Board Can. 32: 233–242.

Les auteurs ont suivi, par dépistage aux ultra-sons, les déplacements de saumons nerka (*Oncorhynchus nerka*) adultes pénétrant dans la rivière Skeena en 1969 et 1970. Quinze des 18 saumons nerka libérés dans le cours inférieur de la rivière ont semblé entrer et sortir passivement avec le flux et le reflux. Deux poissons ont remonté le cours d'eau indépendamment de la marée, et un a nagé à contre-courant tant au flux qu'au reflux. Pendant ces deux années d'opération, les vitesses par rapport au sol ont été de 1.6 km/h à marée montante et de 2.1 km/h à marée descendante, de sorte que les poissons ont été entraînés en aval à raison de 3 km environ par cycle de marée. Trois saumons libérés en eau salée au large de l'embouchure de la rivière ont également semblé se déplacer passivement avec les courants de marée. Les vitesses par rapport au sol pendant le reflux (3.6 km/h) ont été également plus grandes que pendant le flux (2.0 km/h), indiquant un déplacement net vers le large. Nous en concluons que ces mouvements passifs ne sont pas artefact, mais que les saumons nerka ralentissent et s'arrêtent un temps au moment où ils atteignent la «rivière natale», et dérivent pendant quelque temps dans les courants de marée de l'estuaire et de l'embouchure de la rivière avant la remontée.

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THE Skeena River supports a sockeye salmon (*Oncorhynchus nerka*) population of 1–2 million adults, of which 0.5–1.2 million are taken annually in the fishery. Although timing of entry and escapement into the river have been monitored since 1944 by fishing operations (Aro and McDonald 1968; Takagi and Smith 1973), little is known about migration patterns and routes through the estuary and river. This paper reports on sockeye salmon movements in the Skeena River estuary during the 1969 and 1970 runs as revealed by ultrasonic tracking techniques.

The Skeena flows into the ocean through Inverness, Marcus, and Telegraph passages. Depending on the tides, silt-laden water may extend from

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Lelu Island to Lawyer Islands and down Telegraph Passage to Marrack Island (Fig. 1A and B). Tidal influence is noticeable 50 km upstream from the river mouth. This freshwater–saltwater interphase will be considered the estuary.

Salt water wedging into the river along the bottom during flood tide causes fresh water over it to rise and flow back inland. Strongest upstream currents occur around high tide, especially in river channels near Veitch Point and Tyee (Fig. 1B). One hour after high water the current in the lower river turns, and the strongest flows downstream are evident during the last half of ebb tide.

Skeena River water is very silty; secchi disc visibility ranges from 0.15 to 0.9 m during both high and low tides. Silt deposition has created shoals, some of which dry at low tide.

Sockeye salmon enter the Skeena River on their way to a number of spawning areas between early June and late August with a peak during the latter part of July. Sonic tracking operations in 1969 and 1970 were performed from the second week in July until the first week in August. Since Babine sockeye form about 90% of the total Skeena run (Aro and McDonald 1968) and show peak movements through the estuary during the latter part of July, most fish used for tracking must have been of Babine origin.

Materials and Methods

Adult sockeye used for sonic tracking operations in 1969 and 1970 were caught in gillnets. Fish were removed from the net as soon as possible. Sockeye captured near Tyee (Fig. 1A) were penned in the river. In general, tracking started within 12 h after capture; on five occasions fish were held overnight. For tracks that started off the river mouth, fish were held in a tank on board ship. Water in the tank was changed regularly and in all cases the fish was released the same day. When more than one fish was available, the most active was chosen without preference for males or females.

Ultrasonic tags (SR-69, 70 kHz ± 1.5 kHz, pulse rate 4 and 2 pulses/s, Smith-Root Electronics, Seattle) were introduced into each fish's stomach with the transducer pointing aft. In addition, a small plastic tube, 50 mm long and 3 mm in diameter, containing instructions for the finder in the case of recapture in the fishery or on the spawning grounds, was attached to the dorsal fin with a small alligator clip. Tagging operations were performed with the fish held underwater. No anesthetic was used.

Fish were kept after tagging for 15–20 min until they looked reasonably recovered and then released by being dipnetted overboard. An SR-70 hydrophone and a TA-60 receiver (Smith-Root, Seattle) were used to get the salmon's direction. For position plots (at 30-min intervals) we moved close to the fish and determined the position of the boat with either a hand-held compass (1969) or radar (Furuno FRA 10 with a 32-km range) (1970). A depth sounder (Ross, 73-m range) was used as a double check by comparing compass or radar locations with depths indicated on the hydrographic charts.

The Skeena estuary is a difficult area for tracking. Fast currents, sandbanks, and mud flats make operations at night dangerous and often impossible. Therefore most tracks were confined to daylight hours. Sound transmission in an area with great turbulence and often distinct saltwater–freshwater interfaces is complex. These conditions interfered with signal reception and greatly hampered fish location, resulting in repeated losses of signal and short tracks.

A number of environmental parameters such as tide, wind direction, wind speed, and precipitation were recorded at each position fix. Cloud cover and sun visibility were noted only when there was a change in the conditions. In two instances a drogue was released during the track to obtain more precise information on water movements. Times of high and low water in the Skeena River were calculated from Prince Rupert tide tables by adding 65 min for low and 20 min for high tide. Turning of the current is taken as 1 h after high and low water.

Results

In 1969, 10 sockeye salmon released in the lower river near Tyee and Veitch points (Fig. 1B) were followed for a total time of 71 h over a distance of 129 km. Tracks lasted between 2.0 and 13.7 h (median 7.1 h) over distances of 5.8–26.1 km (median 12.9 km) (Table 1).

During 1970, 11 sockeye were tracked; three were released in the outer estuary near Hazel Point, four in the lower river at Veitch Point, and four upriver at Tyee (Fig. 1B). These 11 fish were followed for a total of 89 h (median 8.8 h, range 0.6–32.8 h) over a distance of 201 km (median 18.3 km, range 0.9–85 km) (Table 2).

Tracks were terminated mainly because of loss of signal or equipment breakdown; only in one case was the effort abandoned. The 18 salmon released in the river are discussed separately from the three that started from Hazel Point just off the river mouth.

River Tracks

Figures 2A, B, C and 3A and B show routes followed by sockeye salmon in the lower Skeena River. Most movements are restricted to deeper channels. Often sudden changes occured in direction, which invariably corresponded with reversals in tidal flows. The tracks show that in most cases up or downstream movements coincided with rising and falling tides, respectively. A drogue released during track 4-70 at 1 m below the surface (Fig. 3B) moved at identical speed (aver-

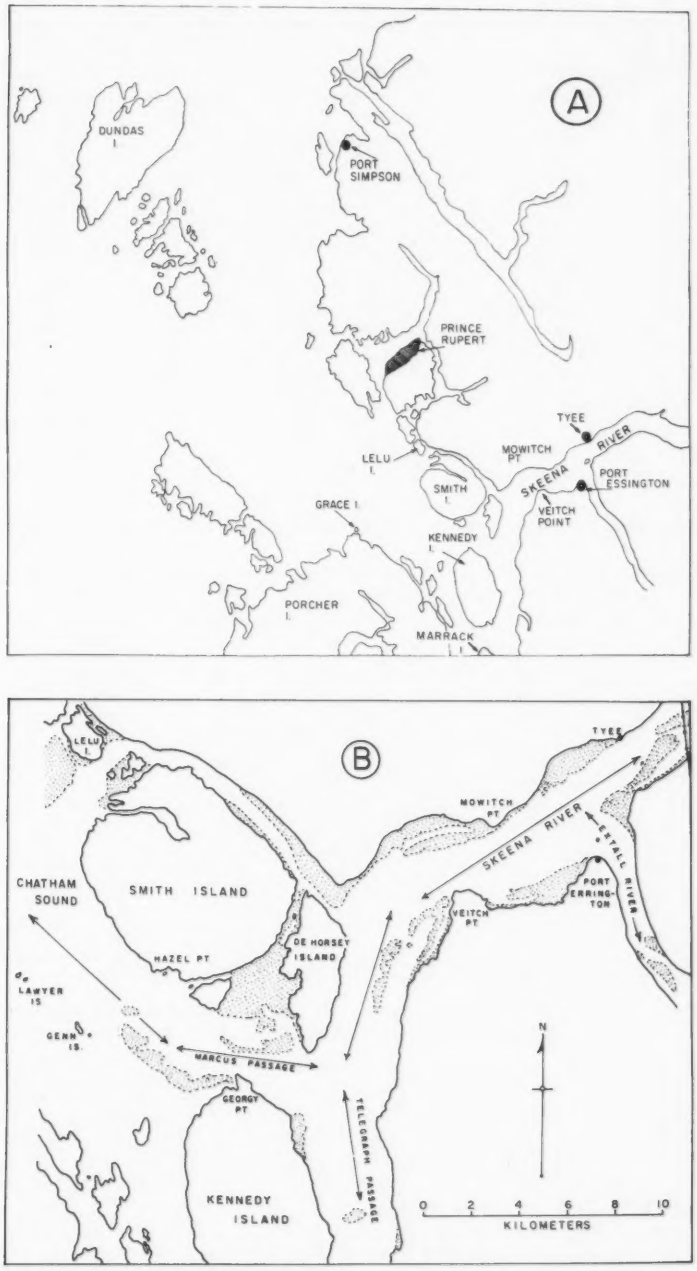

FIG. 1. Skeena River estuary. A, The area around Prince Rupert in northwestern British Columbia, Canada, with the entrance to the Skeena River. B, The Skeena estuary with approximate current directions during ebb and flood indicated by arrows (from hydrographic charts). Grey areas represent shoals.

TABLE 1. Ultrasonic tracking of sockeye salmon (*Oncorhynchus nerka*) intercepted during spawning migrations in Skeena River estuary, 1969.

Track no.	Date caught	Place of capture and release	Released Date	Released Time	Duration track (*h*)	Distance track (*km*)	Avg speed (*km/h*)	Sex
FS- 1-69	July 11	Tyee	July 12	07:55	13.7	16.48	1.20	?
FS- 2-69	July 16	Veitch Pt.	July 16	14:20	9.3	26.08	2.80	♀
FS- 3-69	July 23	Tyee	July 24	04:12	6.2	7.63	1.23	♀
FS- 4-69	July 25	Tyee-Raspberry Is.	July 25	06:46	8.1	15.46	1.91	♂
FS- 5-69	July 27	Tyee	July 28	06:05	4.2	5.86	1.40	?
FS- 6-69	July 28	Tyee	July 29	08:20	4.7	9.44	2.01	♀
FS- 7-69	Aug. 1	Tyee	Aug. 2	06:00	2.0	1.88	0.94	♀
FS- 9-69	Aug. 5(?)	Veitch Pt.	Aug. 5	13:49	5.2	16.54	3.18	♀
FS-10-69	Aug. 6	Tyee	Aug. 7	06:25	5.8	13.05	2.25	?
FS-11-69	Aug. 9(?)	Port Essington	Aug. 9	07:45	11.8	16.34	1.38	?
Totals								
10 tracks — July 11–Aug. 9					71.0	128.76	1.81	

TABLE 2. Ultrasonic tracking of sockeye salmon intercepted during spawning migrations in Skeena River estuary 1970.

Track no.	Date caught	Place of capture and release	Released Date	Released Time	Duration track (*h*)	Distance track (*km*)	Avg speed (*km/h*)	Sex
FS- 2-70	July 8	Hazel Pt.	July 8	09:55	5.5[a]	16.11	2.93	♂
FS- 3-70	July 10	Hazel Pt.	July 10	09:55	3.6	17.86	4.96	♀
FS- 4-70	July 11	Veitch Pt.	July 11	12:35	9.1	23.00	2.53	♀
FS- 5-70	July 15	Veitch Pt.	July 15	14:07	8.7	8.65	0.99	♂
FS- 6-70	July 16	Veitch Pt.	July 16	14:45	11.0	30.56	2.78	♀
FS- 7-70	July 22	Veitch Pt.	July 22	14:35	0.6	0.97	1.62	♀
FS- 8-70	July 23	Tyee	July 23	13:53	0.6	0.88	1.47	♀
FS- 9-70	July 24	Tyee	July 24	15:53	8.6	11.83	1.38	♂
FS-10-70	Aug. 1	Tyee	Aug. 1	17:34	4.9	4.40	0.90	?
FS-11-70	Aug. 2	Tyee	Aug. 2	19:45	4.3	1.53	0.36	?
FS-12-70	Aug. 6	Hazel Pt.	Aug. 6	13:25	32.8	85.28	2.60	?
Totals								
11 tracts — July 8–Aug. 7					89.4	201.07	2.25	

[a]Total track was 10.1 h long but only the last 5.5 h (1430–2000) had useable positions.

age 3.0 km/h) and in the same direction as the fish. Movements in the lower river were therefore rather passive.

A few sockeye salmon did not follow this more or less passive pattern with the tidal currents. For example, fish 9-69 (Fig. 2C) released near Veitch Point more than an hour before low slack, moved upstream at 3.5 km/h and continued this movement for another 4.1 h at 3.2 km/h upriver after the tide changed. Sockeye 10-70 (Fig. 3A) showed the same pattern as 9-69; it moved upriver against the ebb current upon release for about 3.9 h at a speed of 0.5 km/h and continued this upstream movement when the tide changed from ebb to flood for another 1.1 h at a speed of 2.2 km/h. A drogue released during the latter part of this track indicated that fish 10-70 indeed swam against the current before the tide turned (Fig. 3A). Track 9-70 (Fig. 3A) is interesting because this sockeye swam downriver during the rising tide (0.6 km/h for 3.6 h) and then upriver during the falling tide for 5 h at a speed of 2 km/h. Fish 6-70 (Fig. 3C) released during ebb near Veitch Point quickly moved with the ebb flow into Telegraph Passage. When waters started to rise it reversed its movement briefly, but then generally milled around in the same area until the next ebb tide. This sockeye must have resisted flowing back into the lower Skeena River during flood tide.

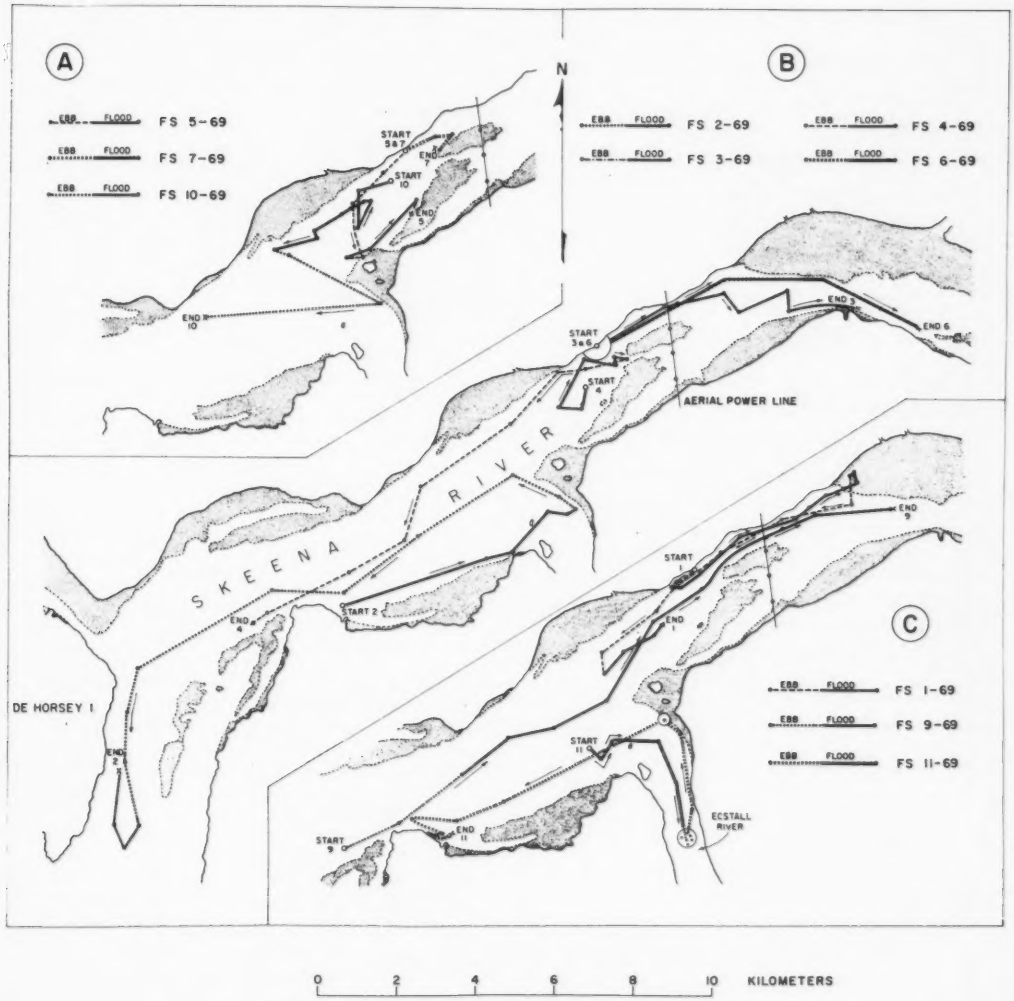

FIG. 2. Sonic tracks in 1969 in the lower Skeena River.

The passive movements indicated in the track patterns were analyzed in more detail by relating a) direction of movement between subsequent position plots to current direction, b) speed of movement to ebb and flood tides, and c) speed of movement to size of tidal oscillation.

The effect of tidal currents on sockeye displacements is illustrated in Fig. 4 and 5, showing that the direction of movement of the fish was generally with the tides. Such passive movements imply that speeds during ebb should be higher than during flood, since there is a net movement of water downstream. Table 3 shows that ebb and flood speeds during both 1969 and 1970 were, respectively, 2.1 and 1.6 km/h. Sockeye salmon thus seem to lose 0.5 km/h, which amounts to 3 km per tidal cycle or about 6 km/day. This will move them out of the river back to the ocean again if no compensatory movements occur.

The net downstream displacement of river water through a tidal cycle is not known for the Skeena River. We approached the problem indirectly by relating speeds of movement to amounts of tidal difference between low and high water. Drops in

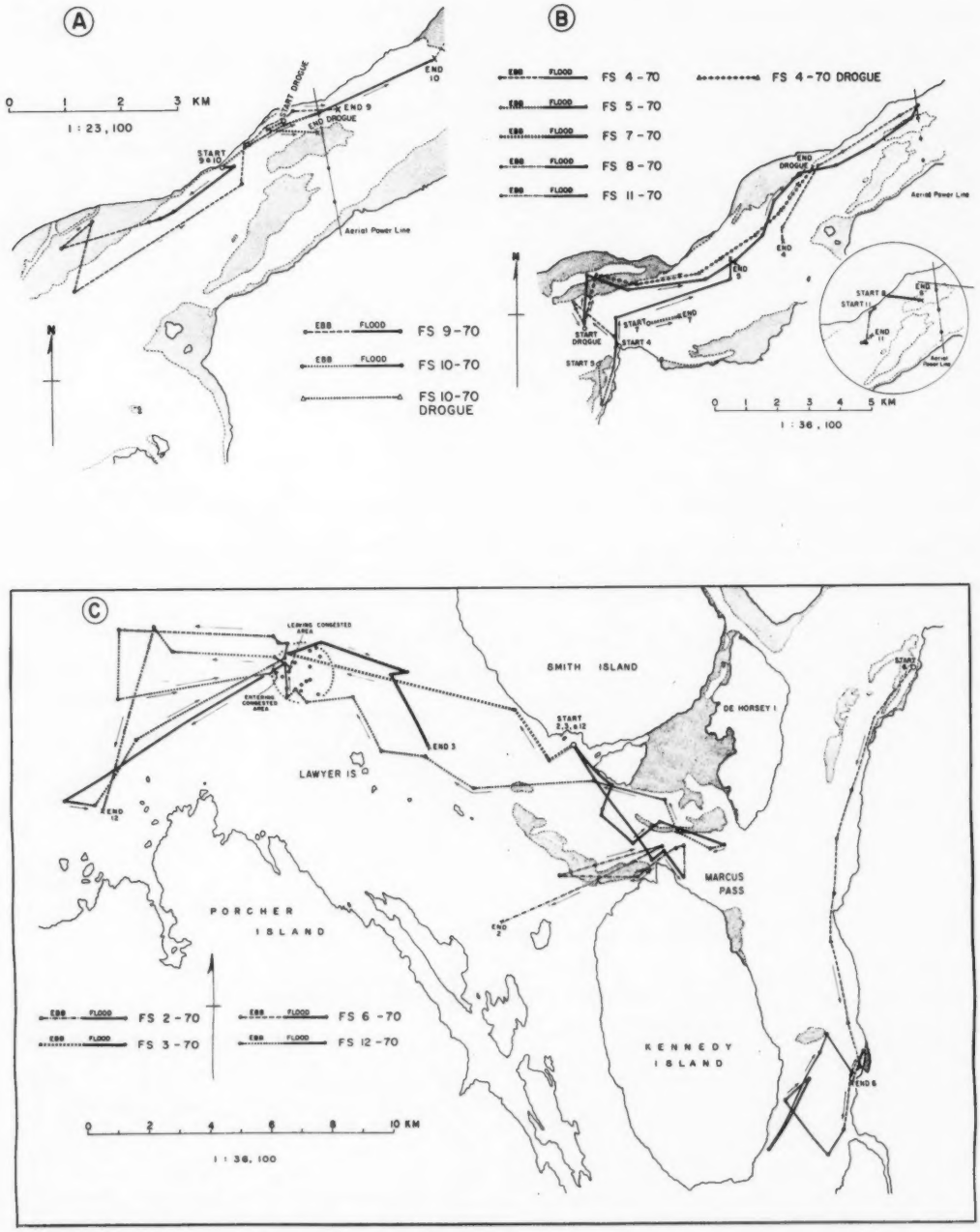

FIG. 3. Sonic tracks in 1970. A and B in lower Skeena River, C in Skeena mouth and offshore.

TABLE 3. Average speed of swimming during ebb and flood in kilometers per hour during 1969 and 1970 sonic tracking operations in the Skeena estuary.

	Ebb			Flood		
Year	Duration (*h*)	Distance (*km*)	Avg speed (*km/h*)	Duration (*h*)	Distance (*km*)	Avg speed (*km/h*)
1969 river	31.4	67.3	2.1	35.8	57.8	1.6
1970 river	15.9	33.3	2.1	27.7	45.1	1.6
1970 ocean	22.4	81.4	3.6	23.4	46.0	2.0

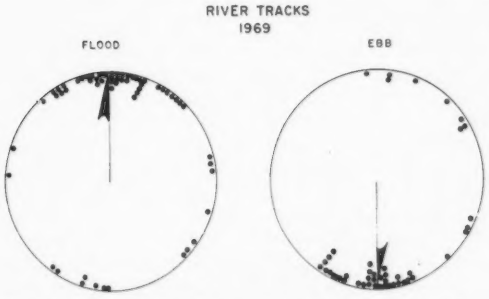

FIG. 4. Directions of movement of sockeye in relation to ebb and flood currents for river tracks in 1969. Arrow from center of circle indicates expected current flow and each dot represents the direction of movement between subsequent position plots relative to the tidal currents.

tidal levels in the Prince Rupert area varied from 1.2 to 6.9 m and rises from 2.4 to 6.1 m during tracking operations in 1969 and 1970. Our assumption was that greater differences in height between high and low water coincide with faster currents up and downstream and should therefore result in greater speeds of movement of salmon. Data points for tracks 9-69, 9-70, and 10-70 are not included in this analysis, because the behavior of these sockeye in relation to tides was different from the majority (see above). Figure 6 shows that ground speeds increased with increasing differences in level between low and high water as expected. Figure 7, however, illustrates that speeds of movements were slower the greater the tide drop. This finding, which is contrary to expectation, may be explained in two ways. It may suggest that sockeye salmon tend to increasingly resist being swept down with the ebb tide, the stronger the current downstream. It may also suggest that the net downstream displacement of river water is greatest when tidal oscillations are smallest. Data collected on the lower Fraser River by D. Hodgins (personal communication) support the latter possibility.

In conclusion, information obtained by sonic tracking in the lower Skeena River indicates that sockeye salmon primarily moved passively up and downstream with the tides. Only 2 out of 18 fish seemed to continually move upstream independent of the tide and 1 always against the current. Upstream movements are necessary to compensate for or counteract net downstream displacement and continued upstream migration must eventually re-

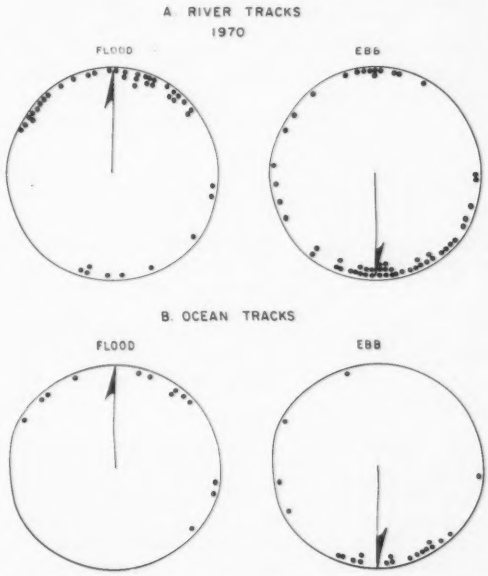

FIG. 5. Directions of movement in relation to ebb and flood current in 1970 for (A) river tracks and (B) ocean tracks (see legend Fig. 4 for further explanations).

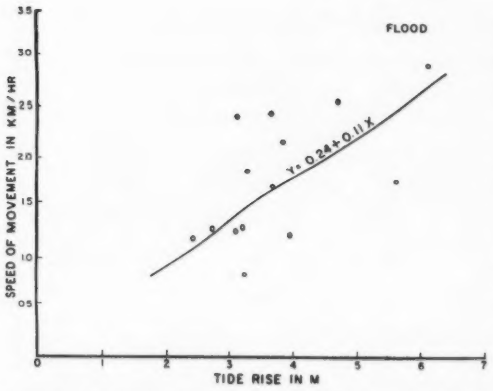

FIG. 6. Ground speeds in kilometers in relation to amount of rise in water level in meters during flood for 1969 and 1970. River tracks.

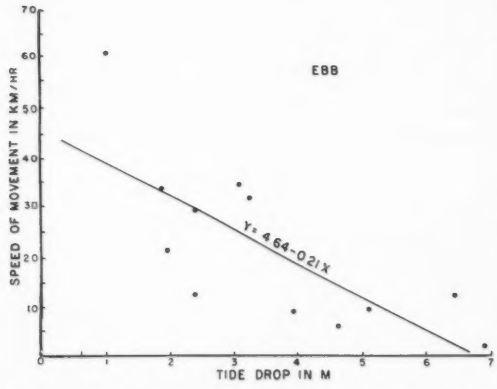

FIG. 7. Ground speeds in kilometers in relation to amount of drop in water level in meters during ebb for 1969 and 1970. River tracks.

place passive movements to reach the spawning grounds.

OCEAN TRACKS

Three sockeye salmon were released outside the Skeena River mouth near Hazel Point (Fig. 1B) during 1970. Tracks 2-70 and 12-70 were started during flood and moved into Marcus Passage until the turn of the tide (Fig. 3C). Both fish moved out to sea with the ebb. The latter swam all the way past Lawyer Island and then seemed to make a number of big circles during the next high and low tides. Fish 3-70 released during ebb moved into Chatham Sound past Lawyer Island and back again on the flood tide towards Marcus Passage (Fig. 3C). In general, movements in open water close to the mouth of the Skeena also seemed strongly influenced by the tides; during falling water the fish moved offshore in the prevailing current direction and with rising water they came in again towards the Skeena mouth. Further offshore in Chatham Sound such clear tidal influence disappeared in the case of track 12-70, which was the only fish followed during a number of tidal periods in salt water.

Speeds of movement during ebb (3.6 km/h) were on the average higher than during flood (2.0 km/h) (Table 3). This relationship also held for sockeye 12-70 during subsequent tidal cycles, although speeds declined with time during both ebb and flood (Table 4). Differences in speed of movement between days one and two were found by C. Groot, C. E. Turner, and K. Simpson unpublished data, during ultrasonic tracking of sockeye in Babine Lake and are probably related to disturbances caused by catching and tagging procedures. In Babine Lake, however, speeds increased with time, while in salt water for track 12-70 speeds decreased.

Discussion

Ultrasonic tracking in the Skeena River estuary revealed that sockeye salmon move primarily with ebb and flood streams. Tracking studies with pink salmon (*Oncorhynchus gorbuscha*) intercepted 70 km south of the Fraser River mouth (British Columbia) also showed some movements which could be explained on the basis of passive drift with flood and ebb tidal currents (Stasko et al. 1973). Since previous tagging and recapture studies in the area indicated no delay in migration of pink salmon, Stasko et al. concluded that the passive behavior of some of their fish was an experimental artifact. This may also apply to our studies with sockeye in the Skeena estuary. However, we consider this unlikely for the following reasons: Madison et al. (1972) tracked sockeye during

TABLE 4. Speed of movement during track 12–70 for subsequent flood and ebb periods.

Time interval	Flood	Ebb
1325–1645	1.5 km/h	
1645–2200		4.5 km/h
2200–0430	2.1 km/h	
0430–1030		3.6 km/h
1030–1600	1.3 km/h	
1600–2230		1.8 km/h

1969 and 1970 near Dundas Island, 93 km north of the Skeena mouth (Fig. 1A), and found that these fish, which were primarily bound for the Skeena River (Aro and McDonald 1968; Shepard et al. 1962), continued to migrate actively after release. Groot et al. (unpublished data) also found active movement after release during sonic tracking operations in Babine Lake with sockeye from the same population, although speeds during the first day of tracking were consistently slower than during the second.

Tagging operations with sockeye and pink salmon in the Strait of Georgia, an inland sea between the mainland of British Columbia and Vancouver Island, indicate that the migration slows and often stops upon reaching the Fraser River estuary (Killick 1955; Vernon et al. 1964; Verhoeven and Davidoff 1962). Gilhousen (1960, p. 42) writes "The delay period of Adams River sockeye of the dominant run is spent in slow wandering movements in southern Georgia Strait apparently within the area where Fraser River discharge is present in the form of a distinct brackish layer at the surface. The sockeye do not approach the Fraser River mouth closely when they arrive off the Fraser Delta. At first they keep to the deeper, clearer waters beyond the edge of the tidal flats . . . . As the time of river entry approaches, the sockeye begin to congregate close to the mouth of the Fraser and to move in on the tidal flats and later into the entrances of the river channels with the flood tide, retreating with the ebb."

Dodson et al. (1972) also report a change in movements of another anadromous fish, the American shad (*Alosa sapidissima*), during its spawning migration in the lower estuary of the Connecticut River. The extensive meandering behavior resulted from a combination of passive drift and relatively inactive swimming. Dodson et al. concluded that the observed meandering does not appear to be a result of handling but may be a consequence of the transition from salt to fresh water.

Other studies confirm that the Skeena sockeye migration slows down as fish approach the river. Fish tagged along the west coast of southeastern Alaska in 1957 and 1958 travelled to the river boundary in 7–10 days, at speeds of 31–44 km/day (Shepard et al. 1962). Aro and McDonald (1968) estimate that mean travel time from Dundas Island to the river boundary is 5 days or about 19 km/day. The distance between Smith Island and Veitch Point of about 18 km is covered between 2 and 5 days (mean 3 days), which averages 6 km/day (Aro and McDonald 1968). Georgy Point and the area east of Smith Island are well known to fishermen where sockeye hold before moving into the Skeena.

Movement into the Skeena River seems related to the tidal cycle (I. Todd unpublished data). Daily catches at Tyee (Fig. 1B) during test-fishing operations in 1968–72 were highest when tidal oscillations were smallest. This pattern was particularly evident during the latter part of July and early August, when upstream migration is at a peak.

The pattern of catches in the commercial fleet also varied with tidal pattern. Maximum catches were recorded near Veitch Point when ebb currents were probably minimal. Catches at this Point were small with maximum ebb, but catches at Standard Point (towards Marcus Passage) were then largest. Little or no commercial fishing for sockeye is conducted in Ogden Channel; this entrance does not appear to be a significant migration route for Skeena-bound sockeye.

Migratory patterns of sockeye salmon in the Skeena River estuary can be summarized as follows. Upon entering Chatham Sound (5 days or 100+ km away from the Skeena River mouth) migration progress slows down and gradually comes to almost a complete halt in the Skeena River estuary. Fish may enter the lower river through the gap between Smith and Kennedy islands on the flood tide and retreat with the ebb into the top end of Marcus Passage. After a period of passively moving in and out with the tidal currents, migration upstream occurs during periods of small tidal oscillations.

Acknowledgments

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Metabolic Studies on the Amphipod *Anisogammarus pugettensis* in Relation to its Trophic Position in the Food Web of Young Salmonids

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CHANG, B. D., AND T. R. PARSONS. 1975. Metabolic studies on the amphipod *Anisogammarus pugettensis* in relation to its trophic position in the food web of young salmonids. J. Fish. Res. Board Can. 32: 243–247.

The marine amphipod *Anisogammarus pugettensis* has been studied as part of the natural food of young salmon and as a potential organism for aquaculture projects. It has been shown to be an omnivore capable of eating seaweed, diatoms, fish pellets, and fish. It survived well under eurythermal and euryhaline conditions but grew best (14% of body weight per day) at a salinity of 28‰ and 20 C. Growth efficiency (K_1) was between 19 and 34%, depending on the size of animal and temperature.

CHANG, B. D., AND T. R. PARSONS. 1975. Metabolic studies on the amphipod *Anisogammarus pugettensis* in relation to its trophic position in the food web of young salmonids. J. Fish. Res. Board Can. 32: 243–247.

Nous avons étudié l'amphipode marin *Anisogammarus pugettensis* comme constituant du régime naturel des jeunes saumons et comme organisme potentiel pour des projets d'aquiculture. L'espèce s'avère omnivore, capable de manger des algues, des diatomées, des boulettes de poisson et du poisson. Elle survit facilement dans conditions eurythermes et euryhalines, mais croît le mieux (14% du poids corporel par jour) à une salinité de 28‰ et une température de 20 C. L'efficacité de croissance (K_1) varie entre 19 et 34%, selon la taille de l'animal et la température.

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THE marine amphipod *Anisogammarus pugettensis* has been described as occurring intertidally and subtidally on the mudflats along the west coast of North America (Barnard 1954; Bousfield 1957). The species is generally found in water of relatively high salinity and may be present in large numbers in the vicinity of pulp mills (Waldichuck and Bousfield 1962). From stomach analyses of young salmonids (Levings 1973) *A. pugettensis* is apparently an important food organism of young salmon feeding close to shore. Little is known, however, about the natural history of *A. pugettensis*, and this report is an attempt to understand its life cycle, metabolism, and food requirements in relation to its habitat.

In addition to the above purpose, however, a need exists to cultivate a suitable food organism for young fish. At present this market is largely occupied by the frozen brine shrimp, *Artemia salina*, which commands a very high price. In earlier studies on a simulated upwelled impoundment (Brown and Parsons 1972) as well as in a salmonid aquaculture project (Powers 1973) it was suggested that a marine amphipod might serve as a "domestic" zooplankter for raising young fish. The biological requirements of such an organism should include an insensitivity of the organism toward change (e.g. salinity or temperature), an ability to reproduce throughout the year, high growth rate and fecundity, an ability to consume various kinds of food, and a demonstrated nutritional adequacy and palatability for young fish. It appeared to us that these requirements could be met in a nearshore organism which is a natural food for fish (Levings 1973) and is abundant along the shoreline of British Columbia throughout the year. Thus our report examines the natural life history of *A. pugettensis* and considers possible use of the amphipod in aquaculture impoundments.

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Methods

Amphipods were collected from mudflats at Crescent Beach, B.C. (49° 04'N, 122° 53'W), an area characterized by intertidal beds of eelgrass (*Zostera marina*) and *Enteromorpha* spp. together

TABLE 1. Growth of *Anisogammarus pugettensis* on different foods at 20 C.

	Head length (*mm*)											No. of animals	
	Weeks												
	0	1	2	3	4	5	6	7	8	9	10	Start	Finish
Enteromorpha intestinalis	0.3	0.4	0.5	0.8	0.8	0.9	0.95	1.05	1.2	1.25	1.25	8	3
Enteromorpha sp.	0.3	0.35	0.6	0.65	0.85	0.95	1.05	1.2	1.25	1.3	1.3	7	4
Chondria decipiens	0.3	0.35	0.4	0.5	0.5	0.6	0.6	–	–	–	–	8	0
Trout pellets (Clark's)	0.3	0.35	0.45	0.65	0.8	0.9	1.0	–	–	–	–	8	0
Fish (frozen turbot)	0.3	0.35	0.45	0.55	0.7	0.85	0.95	0.95	1.1	1.2	1.2	8	2
Diatoms (*Pseudonitzchia* spp.)	0.3	0.4	0.5	0.8	1.0	1.2	1.3	1.3	1.4	1.5	1.5	5	4

with associated species of epiphytic and episammic diatoms. Monthly collections were made at low tide from small ephemeral tidepools or from under clumps of algae cast up on the mudflats. The amphipods were collected using large bore bulb pipettes, small nets, or by hand. Approximately 100 live animals were collected each month; these were measured and sexed in the laboratory and examined for ovigerous females.

Animals were fed six different foods (Table 1). Feeding experiments were carried out in clear plastic compartmentalized trays with individual animals in about 100 ml of filtered sea water (28‰). Illumination was provided by fluorescent lighting for 13 h/day and the temperature was maintained at 10 or 20 C. Following the weekly size measurements, animals were transferred to fresh sea water and provided with excess food.

Growth rate of live animals was most conveniently measured from head length which was then related to dry weight in a separate study. Oxygen consumption was measured with YSI Model 53 oxygen electrodes and the oxygen uptake converted to carbon consumed per animal, assuming RQ of 1.0. The oxygen level in the closed vessels was not allowed to drop below 70% saturation. Metabolism is reported as a percentage of body weight.

Assimilation was determined by a radioactive tracer technique using ^{14}C labelled *Enteromorpha intestinalis* (see Edmondson and Winberg 1971). Only large animals were used in these experiments because of the difficulty of collecting fecal material from the juveniles. Radioactivity of the animals was measured after short feeding periods (48–96 h); radioactivity of fecal pellets and any soluble organic carbon was also measured. Assimilation was then calculated from the activity of the animals divided by the sum of the activities of the solid fecal material, soluble carbon, and the animals. The method tends to underestimate true assimilation by small amounts of assimilated carbon that may be respired or excreted directly from the radioactive food eaten.

Dry weight to carbon conversions were made assuming a factor of 0.5, and growth efficiency (K_1) was expressed as the total food for growth divided by the total food eaten. Ingestion was estimated from the data on assimilation, respiration, and growth.

Results and Discussion

Anisogammarus pugettensis occurred in the intertidal waters of Crescent Beach throughout the year from May 1973 to August 1974. There was no indication of seasonal dominance of either immature or adult forms. The average ratio of adult males to females was close to unity and the presence of young juveniles or females carrying eggs was observed every month except January 1974, when no samples were taken. Thus, *A. pugettensis* appears to breed and grow throughout the year although its growth rate is obviously greater during periods of warmer temperature (Fig. 2). The life cycle of *A. pugettensis* is in contrast to that of beach amphipods (Fincham 1971) and several species of intertidal amphipods (Steele and Steele 1969, 1970, 1972) found in colder North Atlantic environments. The latter organisms have been shown either to have a single brood per year or to enter a resting stage in their reproductive cycles for several months during the winter.

Seawater temperatures and salinities of the animal's habitat observed during sampling ranged from 5 to 22 C, and from 20 to 28‰, respectively. From additional observations it appears that the animal cannot survive in fresh water but may tolerate salinities as low as 11‰ for at least 7 days. The animal's tolerance to low oxygen levels has not been determined precisely. However, in laboratory experiments animals survived oxygen levels of 20% saturation for several hours but did not survive in the absence of oxygen.

Among the six different foods, the seaweed *Chondria* and the fish pellets did not support growth to adulthood (Table 1). However, fish pellets supported a growth rate comparable to that of other foods up to the sixth week, while the animals fed *Chondria* showed a depressed growth rate throughout the experiment. The latter effect may have been due to the relative toughness of the *Chondria* tissue and the growth shown may have been due to organisms living on the *Chondria*. The reason for the high mortality of young animals fed fish pellets was not investigated; however, fouling of the culture medium in the presence of fish pellets may have contributed to the animals' mortality. In all experiments it was observed that the cause of individual mortality was not always the same. Two causes which were independent of food offered were difficulties in molting and if young animals became attached to the surface film they were often unable to return to the culture medium and could die from exhaustion or starvation.

Two species of *Enteromorpha*, frozen fish, and diatoms supported recently hatched young to adulthood. With *Enteromorpha*, the young survived and grew best when given young thalli. Growth rate, final size, and survival of animals fed the diatom culture were all higher than animals fed the other food types. However, nutritional requirements of this animal can be met by a variety of foods, including both animal and plant material. The animal thus appears to be an opportunistic feeder which would favor its use in mariculture impoundments where it could dispose of dead fish and uneaten fish food as well as

lithophytic diatoms and larger algae which might accumulate.

The metabolic requirements of different sized animals at 10 and 20 C are shown in Fig. 1. These curves appear similar to those showing established relationships between body size and metabolic requirements. The Q_{10} value of approximately 1.6 appears lower than an expected value for microcrustaceans of about 2 (Ikeda 1970). However, this may reflect an evolutionary adjustment to repeated temperature change in the animal's intertidal habitat.

The average assimilation value calculated for eight large (10 mg) animals was 59% with a range of 47–72% when feeding on *E. intestinalis*. Due to the quite different and more fragile structure of diatoms (which appear to be the animal's preferred food — Table 1) it is possible that the assimilation efficiency calculated for *Enteromorpha* is lower than for other foods.

The growth rate of animals at 10 and 20 C is shown in Fig. 2. Maximum growth rates of 4.1 and 14.3% per day at 10 and 20 C, respectively, are very similar to the growth rate of the principal pelagic calonoid copepod (*Calanus plumchrus*) found in the Strait of Georgia (Parsons et al. 1969). The Q_{10} of about 1.6 for metabolism is appreciably less than the animal's Q_{10} for growth (about 3). Thus the animal appears to have a low metabolic response to daily (tidal) changes in temperature but can take full advantage of a persistent increase in temperature in order to grow.

Assuming a constant assimilation efficiency, the growth efficiency (K_1) for larger animals is apparently greater than for smaller animals (Table 2); this observation is consistent with other reports (e.g. Mullin and Brooks 1970 — see Parsons 1974 for discussion). Furthermore, the

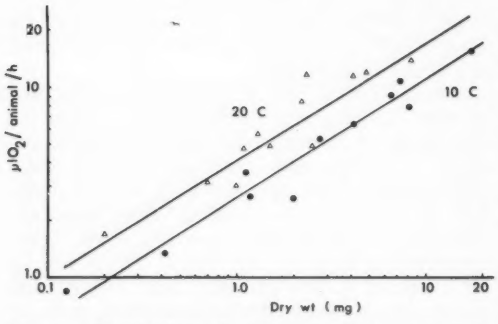

FIG. 1. Respiration of *Anisogammarus pugettensis* at 10 and 20 C.

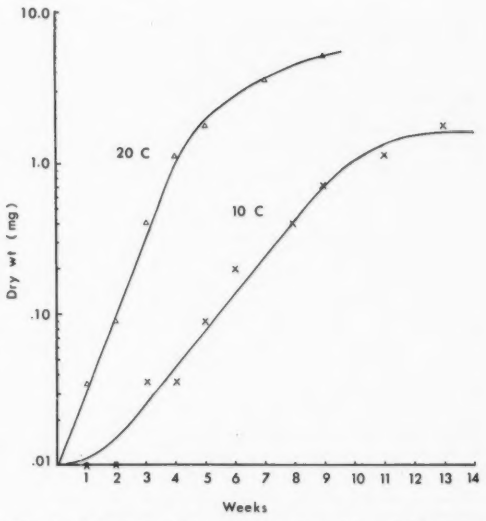

FIG. 2. Growth of *A. pugettensis* at 10 and 20 C fed on *Enteromorpha intestinalis*.

animal grows more efficiently at 20 than at 10 C. This effect is caused by the Q_{10} for growth being larger than the Q_{10} for metabolism. A similar effect was observed by Ivleva (1970) who showed that growth efficiency of the neritic prawn, *Leander adspersus*, increased with temperature from 7 to 15 C; a marked decrease in growth efficiency was then observed at 25 C.

An assessment of food required for reproduction has not been included in Table 2 since the number of young per female as well as the number and frequency of broods appears variable. From laboratory observations, females are known to be able to have at least three broods with between 30 and 90 young per brood. However, ovigerous females brought to the laboratory from the field were observed to release up to about 200 young in a single brood. If the weight of a young amphipod on hatching is about 10 μg, then the possible range of values for the cost of egg production would be from 3 to 15% of the body weight of an adult female.

In conclusion, metabolic requirements of the intertidal amphipod, *A. pugettensis*, appear similar to those of other pelagic microcrustaceans. The principal advantage to the mass cultivation of this organism as opposed to many pelagic species would be its ability to tolerate a wide range of temperatures and salinities and to survive upon different kinds of food. In comparison with the brine shrimp, *Artemia salina*, which has a life

TABLE 2. Metabolic requirements of *A. pugettensis* feeding on *Enteromorpha intestinalis*. (Assimilation is based on average value for large animals at 10 C.)

Animal wt (*dry wt mg*)	% of body wt/day			Assimilation (%)	Total food intake (% *of body wt/day*)	Growth efficiency (K_1%)
	Metabolism	Growth	Total			
0.5 (20 C)	13.2	14.3	27.5	59	46.6	31
0.5 (10 C)	8.4	4.1	12.5	59	21.2	19
1.0 (20 C)	10.2	14.3	24.5	59	41.5	34
1.0 (10 C)	6.0	4.1	10.1	59	17.1	24

cycle of 14 days at 28 C (Teramoto and Kinoshita 1961), the growth rate of *A. pugettensis* appears slow. However, the cultivation of *Artemia* in temperate latitudes would require the extra expense of providing a warmwater environment. Thus, the potential for raising *A. pugettensis* throughout the year in a nutrient enriched upwelled environment (e.g. Brown and Parsons 1973) appears a distinct possibility which is currently being researched.

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Selenium Determination on Fish Tissue

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BEAL, A. R. 1975. Selenium determination on fish tissue. J. Fish. Res. Board Can. 32: 249–252.

A wet-digestion procedure for selenium analysis of fish is described. It involves digestion of the fish tissue homogenate in a nitric–perchloric–sulfuric acid mixture and subsequent complexing with 2,3-diaminonaphthalene. Interfering elements are masked with disodium (ethylenedinitrilo)-tetraacetate prior to complexing. The slope of standards is consistent and recoveries range from 85 to 100%.

BEAL, A. R. 1975. Selenium determination on fish tissue. J. Fish. Res. Board Can. 32: 249–252.

Nous décrivons une méthode d'analyse, par digestion à l'état humide, du sélénium chez les poissons. Le mode opératoire comporte la digestion d'un homogénat de tissu du poisson dans un mélange d'acides nitrique–perchlorique–sulfurique, suivie d'une complexation avec du 2,3-diaminonaphthalène. Les éléments en interférence sont masqués avec du (éthylénedinitrilo)-tétraacétate disodique avant la complexation. Les étalons ont une pente uniforme, et la récupération varie entre 85 et 100%.

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GOVERNMENT concern about levels of heavy metals in fish and fish products led to a request for the Inspection Branch to develop a selenium analysis procedure. Following a literature search and subsequent experimentation, a wet-digestion method and subsequent complexing with 2,3-diaminonaphthalene (DAN) for fluorescence measurement was developed.

To convert organically bound selenium to the inorganic IV oxidation state, biological material must be subjected to strong oxidizing conditions. Many investigators (Ganther et al. 1966; Grant 1963; Grant et al. 1961; Kelleher and Johnson 1961; Hoffman et al. 1968) have used a nitric–perchloric acid mixture for digestion of plant and animal materials with little or no losses. This mixture dissolves large samples per unit weight and, with its high oxidation potential, reduces the chances of charring and volatization losses. The maximum temperature of this mixture was 215 C when monitored throughout the digestion process on a Corning PC-100 hot plate set at 4½, thus reducing losses (Grant 1963; Hall and Gupta 1969) which occur at higher temperatures. Others (Grant et al. 1961; Bock et al. 1964; Cukor et al. 1964) have used combustion methods. However, acid digestion requires less time, allowing more samples to be completed (Ewan et al. 1968). In some cases (Watkinson 1966) HCl is used to reduce Se VI to Se IV which reacts specifically with several reagents. Disodium (ethylenedinitrilo)-tetraacetate (E.D.T.A.) is used to mask interference from ions such as copper and iron (Watkinson 1960) while NH_2OH is added to prevent oxidation of DAN and eliminate traces of HNO_2 (Lott et al. 1963).

Primarily, two complexes are utilized for fluorescence measurements of selenium, one being 4,5-diaminophenylpiazselenol (naptho-[2,3-d]-2-selena-1,3-diazole) and the other 3,4-diaminophenylpiazselenol (Parker and Harvey 1962). The former, from DAN and selenite, is extractable from acid solutions into organic solvents. A number of papers (Allaway and Cary 1964; Cukor et al. 1964; Lindberg and Siren 1965; Lott et al. 1963; Parker and Harvey 1962; Watkinson 1966) indicate preference for DAN. This reagent can be purified by extraction immediately prior to use, but is light sensitive and should be prepared, stored, and used in dim or yellow light.

Solvents used to extract the fluorescent complex include cyclohexane, decalin, toluene, and petroleum spirits. The cyclohexane extract with a fluorescence maximum at 520 mμ and the decalin extract with a maximum of 560 mμ are most suitable (Parker and Harvey 1962).

A method involving wet digestion resembling

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TABLE 1. Recovery of added selenium from trout fillets.

Sample	Trials	Se added	Se found	% recovered	Variance	SD
Trout fillet 1 g	11	0.0 μg	.21 μg[a]	0	.0004	.020 μg
Trout fillet 1 g	11	0.2 μg	.39 μg[a]	90	.0011	.034 μg

[a]Average value.

that of Hoffman et al. 1968, is described here. It is rapid and affords analysis of 10–12 samples per day by one technician, with a sensitivity of 0.6 ppb for a 1-g sample.

Methods

One to 2-g samples of ground fish tissue are weighed into 125-ml Erlenmeyer flasks which, along with all other glassware, has been washed in a 1:1 nitric–sulfuric acid mixture. Four or five glass beads and 6 ml conc. HNO_3 are added and the contents boiled until the organic matter has broken down. The flasks are removed and cooled before 2 ml $HClO_4$ conc. and 5 ml H_2SO_4 conc. are added. Boiling is continued on a hot plate in a perchloric acid fume hood until the solution turns green, then water white again. If, after swirling, the green color does not return the samples are removed and cooled. During the digestion small amounts of conc. HNO_3 are added if charring occurs.

After cooling, 1 ml of 30% H_2O_2 is washed down the walls of each flask. The contents are boiled until white fumes appear, then removed and cooled. The above peroxide addition is repeated twice more.

When cool, the contents are diluted by the addition of 20 ml of deionized water.

Ten milliliters E.D.T.A.–$NH_2OH \cdot HCl$ solution and 25 ml of 6 N NH_4OH are added in turn with agitation prior to the addition of 5 ml of DAN solution. The 0.02 M E.D.T.A.–$NH_2OH \cdot HCl$ solution is prepared by dissolving 7.45 g dihydrate and 25 g hydroxylamine hydrochloride in deionized water, then diluting the solution to 1 liter. The 0.1% DAN solution is prepared by dissolving 0.1 g 80 mesh DAN in 100 ml 5 N H_2SO_4. This solution is extracted 3 times with 15-ml cyclohexane using a 125-ml separatory funnel with a glass wool plug as a filter. The solution must be prepared daily and stored in a darkened cupboard. The contents of the flasks are brought to a boil over a bunsen burner, then placed on a hot plate to continue boiling for 2 min.

The flasks are placed in a darkened cupboard for 1½–2 h after which 20 ml of cyclohexane is added to each. The flasks are stoppered with #4 plastic stoppers containing a tiny air hole and then agitated on a Burrell model #BB wrist-action shaker for 5 min. A piece of masking tape across the top will ensure that the stopper does not fall out during shaking. When the layers have separated, the upper organic layer is drawn off with a pasteur pipette. Adding H_2O to bring the organic layer into the neck of the flask will aid this operation. The samples are placed into the appropriate cuvettes (12 × 75 mm matched) which have been washed in ethanol and rinsed with acetone. Then the fluorescence value is read on the fluorometer (Turner model 110 with a primary filter #110-811 (7–60), 360 nm and a secondary filter #110-826 (2A–15), 520 nm in combination with #58 (Canlab F6150-21), 525 nm).

Standards ranging from 0.1 to 0.5 μg are treated in the same manner as the samples. The selenium standard is prepared by dissolving 0.1634 g reagent grade selenius acid (H_2SeO_3) in deionized water and diluting it to 1 liter. The 0.1-mg Se/ml stock is further diluted with 0.1 N H_2SO_4.

Results and Discussion

Standards prepared from either pure selenium powder or selenious acid gave consistent slopes over a 1-mo period, with constant instrument settings and a reagent blank set to zero on the null meter. The regression line equation $y = mx$ gave an average of 161.4 units/μg Se where y = fluorescence units, m = slope, and x = μg selenium. The slope variance was 206.9 while the standard deviation was 14.4 with a range of 141–182.

A recovery study on trout fillets gave 90% recovery for 11 trails on 11 different days (Table 1). Other fish samples yielded recoveries from 85 to 100%. A relation between the weight of sample analyzed and the amount of selenium found appeared when several samples of oyster (dried) were analyzed with different solvent volume (Table 2). It was found that smaller sample weights resulted in higher absolute selenium values, while smaller solvent volumes resulted in less precise results. Sample weights of 1 g and solvent volumes of 20 ml are suggested for the most consistent data.

As this lab was dry ashing fish tissues with a magnesium oxide–magnesium nitrate slurry for arsenic determination, an attempt was made to use an aliquot of this dissolved ash for the selenium determination. However, a recovery trial on an oyster sample treated by wet digestion after ashing resulted in only a 47% recovery (Table 3). Although selenium and arsenic appear in the same

TABLE 2. Selenium found with varying sample weight and varying solvent volume.

Sample wt (*g*)[a]	Solvent (*ml*)	Selenium found (*ppm*)	Avg (*ppm*)
1.0	6	1.91	
1.0	6	1.50	1.54[b]
1.0	6	1.30	
1.0	6	1.45	
1.0	20	1.55	
1.0	20	1.56	1.54
1.0	20	1.47	
0.5	20	1.76	
0.5	20	1.65	1.69[c]
0.5	20	1.67	
0.5	20	1.69	

[a]Dried oyster.
[b]Variance (0.0511).
[c]Higher readings due in part to no blank correction.

TABLE 3. Dry ashing recovery of selenium.

Sample	Wt	No. of trials	Added Se (μg)	Se found (μg)
Oyster	1 g	3	0.00	1.14
Oyster	1 g	1	2.00	2.08

family in the periodic table their oxidation potentials differ and thus they are not retained by the slurry in the same state.

Atomic absorption analysis of selenium thus far has yielded irregular results due to inconsistent evolution of selenium hydride into the hydrogen flame. This may be caused by varying acid strength due to dilution and impurity of the stannous chloride used prior to reduction of the selenium. Further work may prove this method to be superior in the number of samples that can be analyzed in 1 day.

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Comparison of Canadian and Japanese Merchant-Ship Observations of Sea-Surface Temperature in the Vicinity of Present Ocean Weather Station "P," 1927–33

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COLLINS, C. A., L. F. GIOVANDO, AND K. B. ABBOTT-SMITH. 1975. Comparison of Canadian and Japanese merchant-ship observations of sea-surface temperature in the vicinity of present Ocean Weather Station "P," 1927–33. J. Fish. Res. Board Can. 32: 253–258.

A comparison has been made of sea-surface temperatures obtained by Canadian and Japanese merchant vessels in the vicinity of present Ocean Weather Station "P" (50°N, 145°W) from 1927 through 1933. The Canadian data were obtained by thermograph, the presumably more numerous Japanese data by bucket sample. Both data sets clearly display an annual oscillation. Agreement between the sets is good in spite of differences in data density and in methods of observation and of processing. Characteristics (amplitude, phase) of the annual oscillation are more consistent between the sets than is the arithmetic-mean temperature for the period. Heating of water in the condenser intake is suggested as the cause of a slightly greater (0.3 C) mean for the Canadian observations.

COLLINS, C. A., L. F. GIOVANDO, AND K. B. ABBOTT-SMITH. 1975. Comparison of Canadian and Japanese merchant-ship observations of sea-surface temperature in the vicinity of present Ocean Weather Station "P," 1927–33. J. Fish. Res. Board Can. 32: 253–258.

Nous comparons les températures de surface mesurées par des navires marchands canadiens et japonais au voisinage de la Station météorologique océanique «P» (50 N, 145 W) de 1927 à 1933. Les données canadiennes ont été obtenues à l'aide d'un thermographe, et les données japonaises, probablement plus nombreuses, sur un échantillon prélevé dans un seau. Les deux séries de données montrent clairement une oscillation annuelle. Il y a bonne concordance entre les séries, en dépit des différences de densité, et de collection et de traitement des données. Les caractéristiques (amplitude, phase) de l'oscillation annuelle sont plus uniformes entre les séries que la moyenne arithmétique de température pour la période. Un réchauffement dans la prise d'eau du condensateur serait responsable de la moyenne légèrement plus élevée (0.3 C) fournie par les observations canadiennes.

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FROM 1921 to late 1939, regular return voyages were made between Vancouver, B.C., Canada and the Far East by "Empress" steamships *Asia*, *Russia*, *Canada*, and *Japan* of the Canadian Pacific Steamship Company (CPSSC). A voyage averaged about 6 wk. In particular, the Empresses of *Asia* and *Russia*, which plied the most northerly of the routes on an approximately great circle path, passed relatively close to the position of the present Ocean Weather Station (OWS) "P" (50°N, 145°W) when westbound and 75 km to the north when eastbound (Fig. 1).

Commencing in 1923, sea temperatures were recorded continuously on board CPSSC vessels (even during time in port); the instrument utilized was a "mercury-in-steel," "pen-recording" thermograph located in the condenser intake, about 5 m below the sea surface (Patterson 1933). The instruments were checked against standard thermometers at both ends of the voyage; they were believed accurate to, at worst, about ± 0.5 C. Subsequent to 1932, the vessel log books included sea-surface temperature readings which had been

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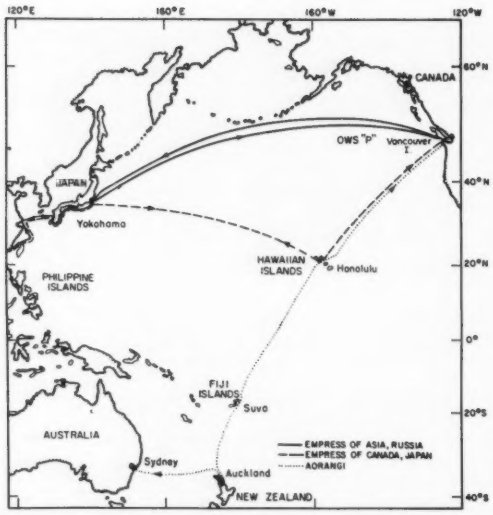

FIG. 1. Regular Pacific Ocean routes for CPSSC and CASSC merchant vessels, 1921–39.

obtained by bucket sampling. All these data are hereafter described by the adjective "Canadian."

There are also available, for the same periods, North Pacific sea-surface temperatures obtained by sampling with canvas bucket from transiting Japanese merchant vessels; the values represent conditions within about the first meter of the surface. The mercury-in-glass thermometers utilized were believed accurate to about ±0.2 C. These Japanese data, averaged by month over "five-degree" squares, form a part of the meteorological and oceanographic information collected throughout the entire North Pacific over a span of many years (Imperial Marine Observatory).

There are limitations in both sets of data. However, a comparison of Japanese and Canadian temperature readings obtained before the present OWS "P" was formally established should provide information upon their value for use in long-term climatological studies of sea-surface conditions in the northeastern Pacific Ocean.

Oceanographic Setting

During autumn and winter, the area in the vicinity of OWS "P" is cold and strongly windswept; these characteristics (together with the effect of conduction and of back radiation) result in a net transfer of heat from sea to air — the "cooling season" (Tabata 1961). As a consequence, strong convection and stirring by wind will occur in the shallower layers of the ocean; in fact, the water becomes effectively isothermal (and isohaline) to depths of the order of 100 m.

During spring and summer, the sea surface is significantly warmed by solar radiation; there is a net heat gain by the sea (Tabata 1961). The presence of this heat, and of stirring by wind, lead to the formation of a "warm" surface layer a few tens of meters thick, bounded below by a "seasonal" thermocline, whose magnitude increases throughout the heating season (Tully and Giovando 1963). In contrast to the cooling season, the surface layer is not generally isothermal. In the absence of wind for a day or more, entrant heat can be confined within a thin layer; this condition can result in a temperature decrease of a few degrees throughout a depth interval of only a few meters. In the presence of wind, any net gain of heat will be mixed downward; the result will be the formation of one or more small, short-lived, thermoclines within the surface layer, providing an irregular "step-structure" in the temperature profile (Tully and Giovando 1963). The heat associated with these thermoclines will in general contribute to the intensification of the seasonal thermocline.

Data Utilized

Canadian and Japanese data from 1927 through 1933 were compared; this choice was dictated by the availability of an effectively continuous monthly record of relevant Canadian thermograph data. Extractions (corrected for instrument error) from the thermograph records provided sea-surface temperature values when the ship's position was effectively north of present OWS "P" (i.e. at longitude 145°W or nearby). An average of 24 values per year was thus obtained throughout the 7-y period, 168 in all. The values were not distributed uniformly by month, although by far the most common monthly value was 2. None could be obtained for 2 mo (February and December of 1933); four were available in 1 mo (March, 1930). Whenever more than one value per month was available, the mean was utilized.

As for the Japanese data, the present OWS "P" is located at the common corner of four adjoining "five-degree" squares. For a more valid comparison with the Canadian data (with, for example, the minimization of any possible effect of the latter's "north-of-P" bias in location), the monthly value used here was taken to be the mean obtained from the two northerly adjoining squares. Each Japanese value therefore represents a "geographical mean" for the area 50–55°N, 140–150°W. Unfortunately, for any given month, measurement of the temperature. Such an effect

information is not available upon either the exact number of Japanese observations or their areal distribution. General knowledge of the maritime activity during 1927–33 indicated that each "monthly" amount of Japanese data was considerably larger than the corresponding Canadian quantity. In addition, if it is assumed that the majority of the Japanese merchantmen plied established trade routes, it would be expected that the sea-surface temperature values would have been obtained in the southerly portion of the five-degree squares considered.

Analysis of Data

Well-defined annual maxima and minima characterized both Canadian and Japanese data; but the Japanese values were generally less (by as much as 1.7 C) (Fig. 2). Maximum temperatures observed coincided in September 1930 for the two sets of data; the Canadian maximum was 15.2 C, the Japanese 13.5 C. The minimum temperature observed by Canadian ships, 3.3 C, occurred in March 1933; the minimum observed by the Japanese, 3.7 C, occurred in February 1931, and also in February 1932.

A sinusoid of the form

$$T_o + T_a \sin(\omega_a t + \phi)$$

was fitted by the method of least squares to the Canadian and Japanese time series. T_o represents the arithmetic-mean temperature for the 7-y period, T_a the amplitude of the annual oscillation, ω_a the frequency (one cycle per year), and ϕ the phase with respect to January 1. The amplitude, phase, and mean were, respectively, 3.5 C, 116°, and 8.1 C for the Canadian data, and 3.7 C, 121°, and 7.7 C for the Japanese data. The characteristics of the annual oscillation (amplitude, phase) differed somewhat less markedly between the two data sets than did the mean. This is characteristic of the northeastern Pacific Ocean; Wyrtki (1965) illustrated that the amplitude and phase of the annual oscillation in the sea surface temperature near OWS "P" changes less with latitude or with longitude than does the mean temperature.

To study further the differences in corresponding monthly-mean values between the Japanese data and the Canadian, the differences (Japanese minus Canadian) were plotted against the Canadian values (Fig. 3). The 82 differences range in value from 1.4 to −2.4 C. About seventy percent were negative, i.e. the (more-near-surface) temperatures obtained by bucket sample were colder.

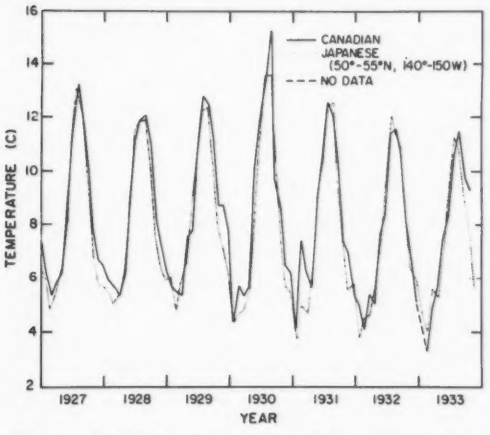

FIG. 2. Monthly-mean sea-surface temperatures "near" 50° N, 145° W (from Canadian and Japanese merchant vessels) vs. time, 1927–33.

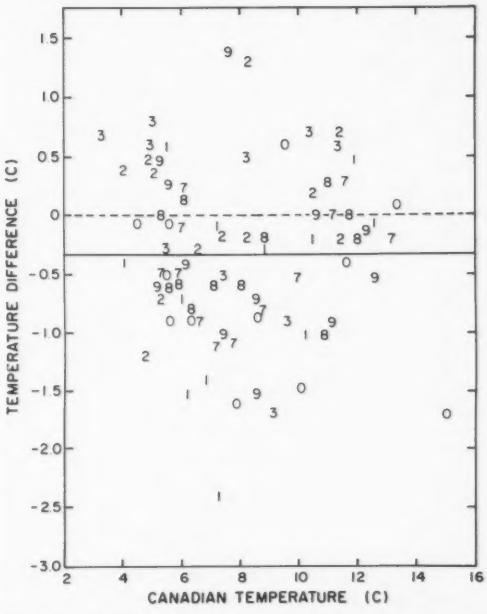

FIG. 3. Monthly-mean sea surface temperatures "near" 50° N, 145° W, from Canadian merchant vessels vs. the difference between monthly-mean sea-surface temperatures from Japanese and Canadian vessels (Japanese minus Canadian), 1927–33. The number associated with each value represents the last digit of year in which the difference was observed, e.g. "0" signifies 1930 and "3" signifies 1933. The solid line at −0.3 C represents the mean difference.

The mean difference was –0.3 ± 0.7 C. The negative values occurred primarily between water temperatures of 5 and 9 C, with a preponderance near the lower temperature. Most positive differences occurred either at temperatures less than 7 C or greater than 10 C (divided about equally between the two ranges); only three such differences were found between 7 and 10 C.

The differences can also be examined in time-series representation (Fig. 4). As shown by large negative values, Japanese temperatures were less than Canadian during autumn and winter, the differences ranging from about 0.5 to about 1.5 C (Fig. 4). This characteristic was less marked during winter 1932–33. During spring and summer, both positive and negative differences were well represented; numerical values were generally smaller than those associated with autumn and winter, often appreciably less than 0.5 C. However in spring and summer 1930, the Japanese temperatures were generally less than Canadian, and the difference was generally as large as was typical for winter.

The seasonal nature of these differences makes them amenable to the sinusoid fitting described above. The resulting amplitude and phase are 0.4 C and 184°. This confirms the marked negative bias that occurred each autumn and winter.

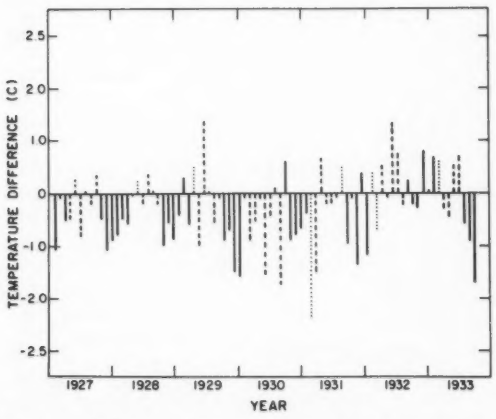

FIG. 4. Difference between Japanese and Canadian monthly-mean sea-surface temperatures vs. time, 1927–33. The length of a vertical line represents the magnitude of the difference. Solid lines represent differences in which both values occurred during a time of decreasing sea-surface temperatures, the dashed lines are differences associated with increasing temperatures. The dotted lines indicate the few remaining cases.

It also indicates a peak-to-peak variation of 0.8 C for the average annual oscillation of the temperature difference.

A final point regarding the temporal nature of the differences (Fig. 4) is that the greatest number of positive temperature differences were recorded during the latter half of the 7-y period, primarily in 1932 and 1933.

Discussion

Sea-temperature data recorded at thermograph depth (5 m) aboard Canadian merchant vessels during 1927 through 1933 have been compared with those obtained throughout the same period by "surface-bucket" sampling from Japanese merchantmen. The data were obtained in the general vicinity of present OWS "P" in the northeastern Pacific Ocean. The monthly-mean values from each of the two groups of data were characterized by an annual oscillation throughout the 7-y period. The results suggest that this oscillation was more consistent between the two groups than was the arithmetic-mean temperature for the period, and appears to be in general agreement with those of more recent work. This accord was present in spite of the fact that the Japanese data were obtained in a different manner than were the Canadian and were, presumably, more numerous.

Some primary characteristics of the differences between corresponding monthly-mean values of "surface-bucket" (Japanese) and condenser-intake (Canadian) temperatures (i.e. Japanese minus Canadian) have been examined. The great majority of these differences were negative; in fact, during times of net cooling in the northeastern Pacific (autumn and winter), the Japanese values were generally appreciably colder by about 0.5–1.5 C). During the remaining times of the year (those characterized by net warming of the sea), it was found that the differences could be negative, positive, or zero; however, whatever the "sign," any non-zero difference was usually smaller than those encountered during autumn or winter.

The most likely cause of such persistent negative differences may be the elevation of temperatures in a ship's condenser intake by conduction of heat from the vessel itself. This warming should indeed always be present to some degree in any vessel. In addition, error may arise from limitations in surface-sampling by bucket. Drawbacks in the use of canvas buckets (i.e. the type employed to obtain the Japanese data) have been studied in some detail by Brooks (1926). A primary source of error was evaporative cooling of the bucket between the sampling itself and the measurement of the temperature. Such an effect

could be expected to be of most significance during cold, windy weather such as that occurring at OWS "P" during autumn and winter. (Since the effect should generally be less, though not necessarily negligible, throughout the remainder of the year, the annual oscillation would be enhanced). Brooks suggested that this and other sources of error can become important if sampling personnel are ill trained and/or poorly motivated. He believed, however, that with due care, quite accurate surface temperatures could be obtained by bucket even during the cooling season. Calculations utilizing values of heat loss from the sea surface during the cooling season (Tabata 1961) suggest that depression of bucket-sample temperatures by the order of 0.5 C from the actual could result from badly executed sampling. Effects of "intake" heating and of bucket-sample cooling would be "additive" with respect to formation of a negative "bucket-minus-intake" difference.

The heating seasons during the study period were marked by much more variety in observational differences. Negative values, generally much smaller than those in the cooling seasons, were often present, although positive or zero differences also occurred. If significant elevation of intake temperatures by heat from the vessel itself is always present, the differences noted could be a function of the oceanic temperature field, contrary to the case in autumn or winter. Thus the magnitude of the differences would depend both upon the strength of the surface heating vis-a-vis that of warming at condenser-intake depth and upon the degree of confinement of this heat above that depth.

Exceptions to this behavior, not readily explainable in the present context, were found occasionally to occur in the differences. We cannot explain the large negative values present during the summer of 1930; the explanation appears to demand detailed knowledge of both oceanographic and atmospheric conditions during, and perhaps previous to, the times in question.

Other studies utilizing far greater amounts of data have reported intake temperatures warmer than bucket temperatures utilizing "same ship" differences. From 6800 "bucket-intake" pairs of observations, Saur (1963) calculated a mean difference of –0.7 C ± 0.9 C. Saur believed that this temperature increase with depth resulted from elevation of intake thermometer temperatures because of incrustation or fouling of the pipe and/or of inadequacies in thermometer installation (the primary cause being conduction of heat to the thermometer through metal fittings). James and Fox (1972) utilized 13,876 comparisons and found the mean to be –0.3 C ± 1.3 C. They also found that bucket temperatures were modified when samples were taken in strong winds or heavy precipitation or when the air–sea temperature difference was large. The choice of bucket type had a bearing on the degree to which environmental influences modified the temperature and it was noted that canvas buckets seemed susceptible to such influences. James and Fox (1972) conclude, however, that a correction program to adjust either intake or bucket temperatures as a function of modifying factors is not feasible.

A number of historical data sets can be combined to yield a time series for OWS "P." The Japanese data extending from 1911 to 1939 are a key element of this time series and based on this study, we are pleasantly surprised by their quality. Collins (1971) reported results of analysis of daily temperature observations at OWS "P" for the years 1959 through 1969. Variations in the yearly mean temperature and the amplitude of the annual oscillation were 1.0 and 0.4 C, respectively. If we consider these results as the expected true oceanic signal, and the Japanese–Canadian differences reported here as "noise," the "signal-to-noise" ratios for the Japanese data would be about 3 and 1 for the yearly mean temperature and annual oscillation, respectively. This encourages study of a combined historical data set for year-to-year variations of mean temperature at OWS "P."

Additional note — From late 1927 to late 1939, the thermograph-equipped S.S. *Aorangi* of the Canadian Australasian Steamship Company (CASSC) made regular return voyages from Vancouver, Canada to Sydney, Australia, via Honolulu, Suva, and Auckland, New Zealand (Fig. 1). Of perhaps historic interest is the fact that two preliminary discussions of oceanographic or meteorological implications deriving from the early thermograph data obtained by the CPSSC and CASSC vessels have been carried out (Patterson and Smith 1929; Patterson 1933).

All "Empress-vessel" thermograph and bucket-temperature records, as well as vessel logs, utilized or mentioned in this paper have been archived on microfiche. Thermograph records from the S.S *Aorangi*, for September 1927 through October 1934 have also been archived although no logs of this vessel have been obtained. Copies of this information can be procured upon request from the Marine Environmental Data Service, Ottawa, Canada.

Acknowledgments

This report has profited particularly from comments by J. F. T. Saur. Valuable criticism was also provided by Drs R. W. Stewart, P. W. Nasmyth, S. Tabata, and J. F. Garrett of the Department of the Environment, Marine Sciences Directorate, Pacific Region, Victoria, B.C.

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Two New Species of *Heterotanytarsus* Spärck, with Keys to Nearctic and Palaearctic Males and Pupae of the Genus (Diptera: Chironomidae)

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SÆTHER, O. A. 1975. Two new species of *Heterotanytarsus* Spärck, with keys to Nearctic and Palaearctic males and pupae of the genus (Diptera: Chironomidae). J. Fish. Res. Board Can. 32: 259–270.

Two Nearctic species of *Heterotanytarsus* are described: *Heterotanytarsus perennis* n.sp. from male imagines, pupae, and fourth-instar larvae; *H. nudalus* n.sp. from imagines and pupae. These are the first published records and descriptions of Nearctic species of this genus.

Keys are given for male imagines and pupae of the four Nearctic and Palaearctic species of the genus.

Heterotanytarsus perennis has been recorded only from Marion Lake, British Columbia. The species is univoltine. *Heterotanytarsus nudalus* has been recorded only from Lake 192, Freshwater Institute Experimental Lake Area, Kenora, Ont. This species is bivoltine. A larva of *Heterotanytarsus* has been found in Sawley Lake, Algonquin Park, Ont. The findings, together with the absence of the characteristic larva from numerous other localities, indicate both species as northern oligotrophic members of chironomid communities.

SÆTHER, O. A. 1975. Two new species of *Heterotanytarsus* Spärck, with keys to Nearctic and Palaearctic males and pupae of the genus (Diptera: Chironomidae). J. Fish. Res. Board Can. 32: 259–270.

Nous décrivons deux espèces néarctiques d'*Heterotanytarsus: Heterotanytarsus perennis* n.sp. à partir d'imagos mâles, de pupes et de larves quaternaires; *H. nudalus* n.sp. à partir d'imagos et de pupes. Ce sont les premières mentions et descriptions publiées d'espèces néarctiques de ce genre.

Nous donnons des clés d'identification des imagos mâles et des pupes des quatre espèces néarctiques et paléarctiques du genre.

Heterotanytarsus perennis n'a été signalé que dans le lac Marion, Colombie-Britannique. L'espèce est univoltaine. *Heterotanytarsus nudalus* n'a été signalé que dans le lac 192, région des lacs expérimentaux de l'Institut des eaux douces, Kenora, Ont. Cette espèce est bivoltaine. On a trouvé une larve d'*Heterotanytarsus* dans le lac Sawley, parc Algonquin, Ont. Ces observations, en plus de l'absence de la larve caractéristique de plusieurs autres endroits, indiquent que les deux espèces sont des membres oligotrophes méridionaux des communautés de chironomides.

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THE lack of knowledge of the specific identities of chironomids taken in bottom samples has hitherto prevented the development of valid lists of indicator communities of lake types such as those known for Palaeartic chironomids (Brundin 1949, 1956b). However, the description of several important members of ultra-oligotrophic, moderately oligotrophic, and mesotrophic communities has made it possible to develop preliminary lists of indicator communities for North America (Sæther 1974b). One of the important genera is *Heterotanytarsus* Spärck of which two new species are described here. There is only one previous record of the genus from North America (Hamilton 1965).

Methods and Morphology

The mounting procedure used is outlined by Sæther (1969, p. 1). The general terminology follows Sæther (1971, 1974a) with the following corrections and modifications discussed by Sæther (1974c). Brachiolum is used instead of basal vein, laterosternite IX is used instead of laterotergite IX, the tarsal "Sinneszapfen" (S2) of Hirvenoja (1973) is called sensillae chaeticae, and stipes is used instead of cardostipes (see Hansen 1973).

Measurements and ratios follow Schlee (1966) with the additions and modifications given by Sæther

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(1969). In the following descriptions measurements are given as ranges followed by a mean when four or more measurements have been made and again followed by a number in parentheses giving the number measured (n).

Holotypes, the allotype of *Heterotanytarsus nudalus* n.sp., and some paratypes have been deposited in the Canadian National Collection (CNC) in Ottawa. Remaining paratypes are retained at the collection of the Freshwater Institute, Winnipeg.

Heterotanytarsus perennis n.sp.

The male imago is characterized by an AR of about 0.5–0.6, wing membrane with 1–16 apical setae, M_{1+2} with 1–3 apical setae, R with 9–13 setae, R_1 with 3–7 setae, R_{4+5} with 7–14 setae, squama with 5–7 setae, and bristle ratio of hind leg 5.9–6.8. The anal point is small, but distinct.

The pupa has a 0.26 to 0.29-mm long thoracic organ, about 4.5–6.6 times as long as wide and without enlarged apical spines; anterior precorneal seta shorter than median precorneal setae and spinelike; three posterior dorsocentral setae 34–58 μ long and spinelike; posterior spines of tergites up to 10–12 μ long; segment VII with 3 filamentous and 1 thinner L-seta and segment VIII with 4 filamentous L-setae.

The larva cannot, for the moment, be separated from that of *H. apicalis* Kieff.

Male Imago (n = 6, Except When Stated Otherwise)

Length 2.10–2.46, 2.27 mm. Wing length 1.33–1.64, 1.49 mm. Total length/wing length: 1.47–1.58, 1.53. Wing length/profemur: 3.26–3.49, 3.42. Coloration blackish brown.

Head — AR = 0.51–0.57, 0.54. Flagellomeres 1–3 with sensillae chaeticae. Outer verticals 4–6, 5; other temporals absent. Clypeus with 7–9, 8 setae. Cibarial pump, tentorium, and stipes as in Fig. 1B. Tentorium 130–140, 135 μ long; maximum width 17–20, 19 μ; width anterior of posterior tentorial pit 9–11, 10 μ; distance from apex to posterior tentorial pit 26–32, 28 μ; stipes 130 μ (2) long. Palp lengths in microns; 20–29, 25; 31–37, 34; 68–90, 81; 66–94, 85(5); 104–130, 114(5).

Thorax (Fig. 1D) — Antepronotum with 4–7, 5 setae. Dorsocentrals 6–9, 8; acrostichals 2; prealars 4–5, 4. Scutellum with 4 setae.

Wing (Fig. 1A) — VR = 1.38–1.55, 1.49. Extended part of costa 22–70, 55 μ long. Brachiolum with 1 seta; R with 9–13, 11; R_1 with 3–7, 5; R_{4+5} with 7–14, 11; M_{1+2} with 1–3, 2 setae. Squama with 5–7, 6 setae. Cell r_{4+5} with 1–10, 3 apical setae; cell m_{1+2} with 0–6, 2 setae. Sensillae campaniformis 6 at base of brachiolum, 3 below seta and 7–8 at apex of brachiolum, 1 on subcosta at humeral crossvein, 1 on Fr, and 1 on R_1 about 100 μ from base.

Legs — Spur of front tibia 34–42, 38 μ long; spurs of middle tibia 18–22, 21 μ and 14–22, 18 μ long; of hind tibia 34–43, 39 μ and 12–18, 16 μ long. Width at apex of front tibia 26–31, 28 μ; of middle tibia 26–33, 29 μ; of hind tibia 30–41, 34 μ. Tarsal pseudospurs absent. No true comb present, but spiniform setae apically on hind tibia end in an irregular apical row of 6–9 setae. Sensillae chaeticae apparently absent on middle leg, 1(2) about one-third from base on hind leg. Lengths (in microns) and proportions of legs are as follows:

	fe	ti	ta_1
p_1	393–472,436	503–619,561	282–337,316
p_2	454–564,510	478–571,527	239–282,262
p_3	405–527,470	527–650,596	288–362,323

	ta_2	ta_3	ta_4
p_1	172–233,206	116–165,148	68–86,78
p_2	141–165,154	98–129,115	57–69,64
p_3	165–202,184	135–161,153	63–80,75

	ta_5	LR	BV
p_1	51–63,59	0.54–0.58,0.56	2.36–2.92,2.66
p_2	51–57,52	0.49–0.50,0.50	3.36–3.50,3.41
p_3	51–63,57	0.53–0.56,0.54	2.89–3.07,2.98

	SV	BR(n = 2)
p_1	3.08–3.20,3.15	3.25–3.53
p_2	3.90–4.02,3.96	4.11–4.27
p_3	3.25–3.36,3.30	5.88–6.80

Hypopygium (Fig. 1C) — Ninth tergum with 4–5, 5 setae at base of the short but distinct anal point; laterosternites IX each with 3–4, 3 setae. Phallapodeme 63–80, 71 μ(5) long. Transverse sternapodeme 57–72, 68 μ long. Gonocoxite 138–174, 163 μ long; gonostylus 66–85, 76 μ long. HR = 2.02–2.22, 2.15; HV = 2.93–3.18, 3.02.

Pupa (n = 2, Except When Stated Otherwise)

Length 3.28–3.56 mm. Length of thoracic horn/length of anal macrosetae 1.00(1).

Cephalothorax — Thoracic horn (Fig. 2D) 264–294 μ long, 40–66 μ wide, 4.45–6.60 times

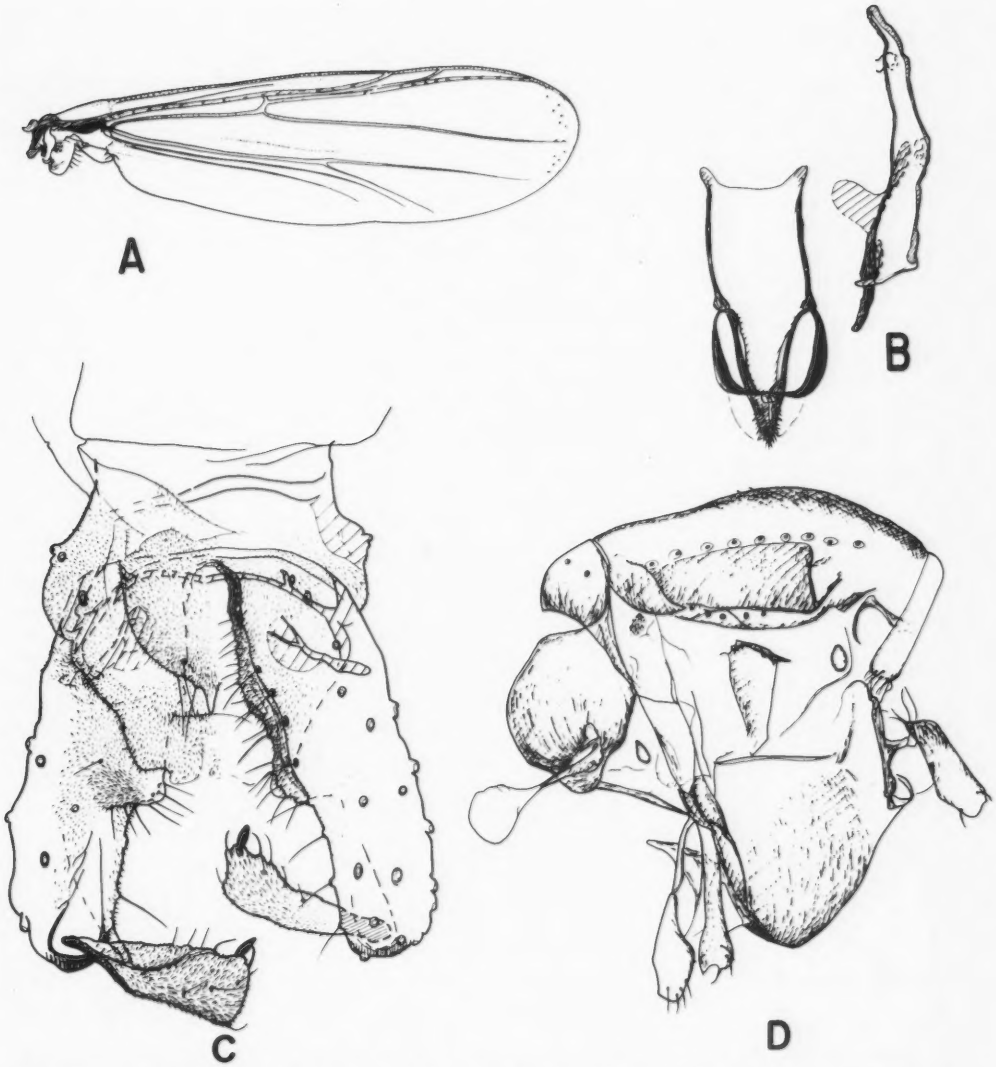

FIG. 1. *Heterotanytarsus perennis* n.sp., male. A, wing; B, cibarial pump, labial lonchus, tormae, tentorium, and stipes (cross-hatched); C, hypopygium; D, thorax.

as long as wide, covered with spines. Anterior precorneal seta (Fig. 2D) spinelike, 31–43 μ long, located 12 μ from median setae and 19–22 μ from posterior seta. Median setae 49–51 μ long, located 5–10 μ from posterior seta. Posterior seta 116–131 μ long, located 58–131 μ from horn. Frontal plate (Fig. 2F) with rugulose warts and 52 μ (1) long frontal setae. Anterior dorsocentral setae (dc_1) 100–160 μ long, located 22 μ from dc_2; remaining dorsocentrals spinelike; dc_2 34–40 μ long located 54–62 μ from dc_3; dc_3 43–58 μ long, located 86–88 μ from dc_4; dc_4 34–40 μ long.

Abdomen (Fig. 2A) — Shagreenation and chaetotaxy as shown in Fig. 2A with 3 filamentous and 1 narrower setae on VII and 4 filamentous

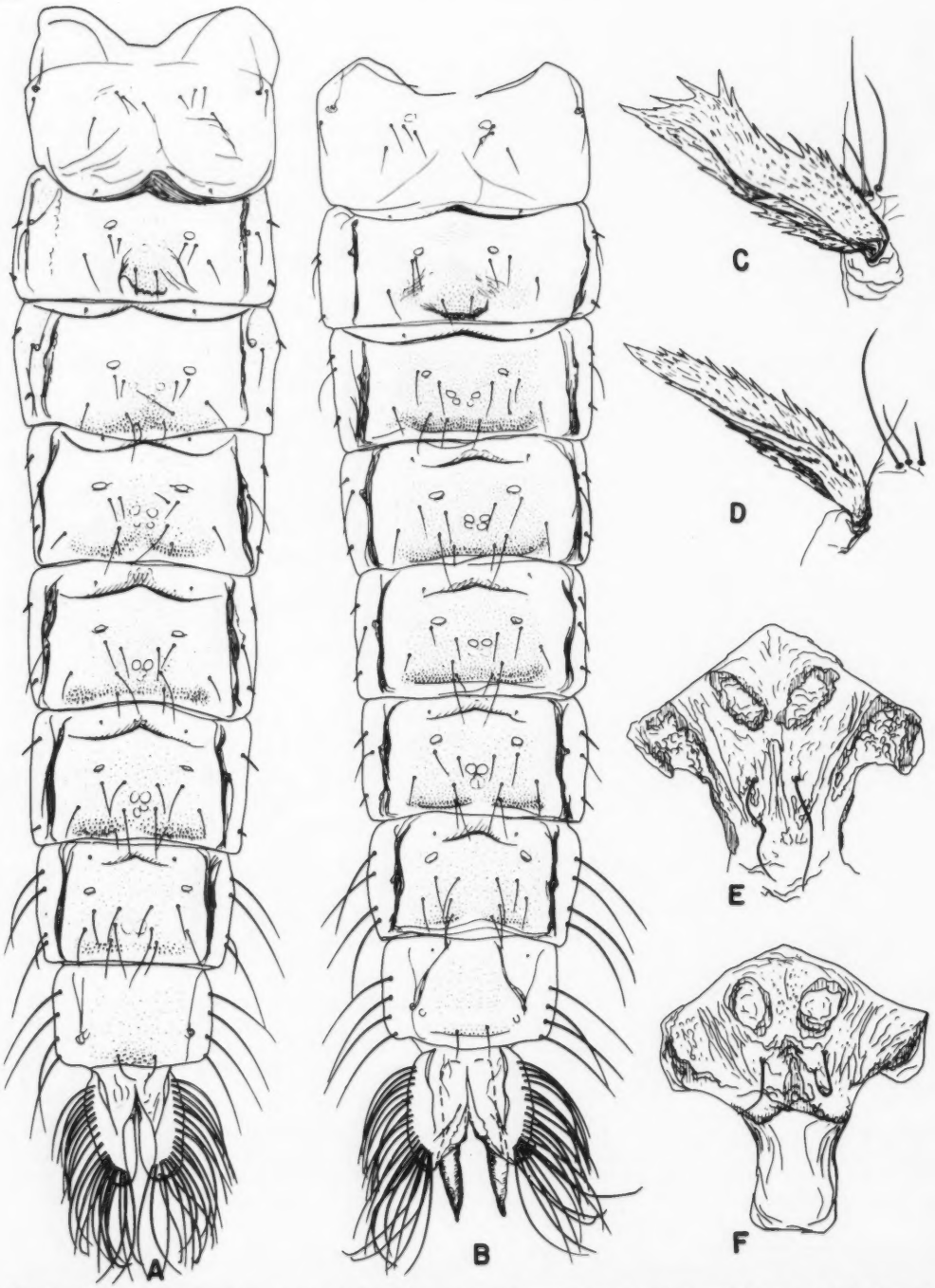

FIG. 2. *Heterotanytarsus* spp., pupae. A–B, abdomen: A, *H. perennis* n.sp.; B, *H. nudalus* n.sp.; C–D, thoracic horn and precorneal setae: C, *H. nudalus* n.sp.; D, *H. perennis* n.sp.: E–F, frontal plate; E, *H. nudalus* n.sp.; F. *H. perennis* n.sp.

setae on VIII. Pedes spurii A present on IV–VII, longest spinules 10–12 μ long on IV, 12–15 μ on V, 9–10, on VI, and 3 μ on VII. Anal lobe with 20–25 setae in fringe, 5–7 of them mediad of the 274 μ (1) long anal macrosetae. Male genital sac with long, fingerlike, apical projection.

FOURTH-INSTAR LARVA (N = 6, EXCEPT WHEN STATED OTHERWISE)

Length 2.84–3.74, 3.21 mm. Head capsule length 0.27–0.31, 0.28 mm.

Head (Fig. 3A) — Antenna as in Fig. 3G. Length of antennal segments in microns; 114–135, 123; 140–163, 147(5); 20–21, 20(5); 9–109(5). AR = 0.67–0.73, 0.70(5). Basal antennal segment 21–24, 23 μ wide; 5.00–5.87, 5.47 times as long as wide; distance from base to annular organ 40–50, 45 μ; to basal mark of seta 8–10, 10 μ (4); to seta at annular organ 46–56, 51 μ (4); to an apparent additional mark of seta 94–100 μ (3); blade at apex 138–167, 146 μ (5) long. Basal Lauterborn organ 18–20, 20 μ (4) long, located 20–26, 23 μ (4) from base; distal Lauterborn organ 22–26, 24 μ long, located 50–55, 52 μ from base. Labrum and palatum as in Fig. 3A, F and in *H. apicalis* Kieff. (Thienemann 1941 fig. 29). Premandible (Fig. 3C) 56–62 μ (2) long. Mandible (Fig. 3D) 108–120 μ (3) long. Mentum (Fig. 3B) with 21–22 μ (2) wide ventromental plate; two median teeth 11–12 μ (2) wide, width of ventromental plate/width of median teeth 1.75–2.00 (2).

Abdomen — Procercus (Fig. 3E) 76–100, 87 μ (5) long; 26–32, 30 μ wide; with 1 strong and 1 minute lateral setae and 6 anal setae 800–979 μ (3) long. Supraanal setae 220–320, 277 μ long. Ratio of length of supraanal setae/anal setae: 0.32–0.40(3). Anal tubules (Fig. 3E) 87–124, 108 μ (5) long; 26–38, 32 μ (5) wide at the enlarged base; 11–22, 18 μ (5) wide at the middle. Posterior parapods 140–149, 142 μ (4) long.

Material Examined

HOLOTYPE: male, Marion Lake, B.C., 14/6/65, A. L. Hamilton (CNC No. 13400). PARATYPES: 5 males, 2 exuviae, 6 larvae, Marion Lake, B.C., April and May 1964, A. L. Hamilton.

Remarks

This species is very closely related to *H. apicalis* Kieff. (Kieffer 1921 p. 806; Spärck 1922 p. 88; Edwards 1929 p. 347; Goetghebuer 1932 p. 90, 1940–50 p. 14; Thienemann 1941 p. 210, 1944 p. 589; 620 Zavřel 1941 p. 23, 1942 fig. 3, p. 19; Brundin 1956a p. 80; Fittkau 1956 p. 13; Pankratova 1970 p. 149). In fact the only differences found between the males of the two species consist in the better developed anal point in *H. perennis*, and in that the outer spur of hind tibia is said to reach only ⅛– of the length of the inner spur in *H. apicalis* (Brundin 1956a p. 81), while it reaches about ½ in *H. perennis*. The descriptions of *H. apicalis*, however, are rather incomplete. The pupa of *H. perennis* has only 3 filamentous (plus 1 additional) L-setae on VII and 4 on VIII, while *H. apicalis* has 5 filamentous L-setae on both segments. Furthermore, the shagreenation in *H. apicalis* is according to Fittkau (1956 p. 19) stronger with the posterior spinules reaching up to 15 μ in length, while in *H. perennis* these spinules at most reach 10–12 μ. Other probable differences consist in the spinelike anterior precorneal setae, the spinelike 3 posterior dorsocentral setae, and in the absence of a small lobe at base of the thoracic horn in *H. perennis*. The larva cannot at the moment be separated from that of *H. apicalis*. It differs from a *Heterotanytarsus* larva from Sawlay Lake, Algonquin Park, Ont., by having a relatively wider ventromental plate and a less wide basal antennal segment.

Heterotanytarsus nudalus n.sp.

The male imago is characterized by an AR of 0.91–1.2, wing membrane without setae, M_{1+2} without setae, R with 3–7 setae, R_1 with 0–1 setae, R_{4+5} with 0–2 setae, squama with 10–17 setae, bristle ratio of hind leg 7.7–8.5, and anal point barely indicated.

The female imago has 0–1 setae on M_{1+2}, none on M_{3+4}, 8–21 setae in cell r_{4+5}, 1–15 in m_{1+2}, 2–4 in m_{3+4}, 6–21 in an, 7–12 setae on the faintly divided tergite IX, and 8–13 setae on gonocoxite IX.

The pupa is easily recognized by the thoracic horn with the very strongly enlarged apical spines and by the presence of 4 filamentous L-setae on each of segments VII and VIII. The thoracic horn is 0.30–0.37 mm long and about 2.8–4.1 times as long as wide.

MALE IMAGO (N = 10, EXCEPT WHEN STATED OTHERWISE)

Length 2.42–3.35, 2.84 mm. Wing length 1.42–1.89, 1.67 mm (12). Total length/wing length: 1.59–1.81, 1.69. Wing length/profemur: 3.40–3.68, 3.57 (11). Coloration blackish brown.

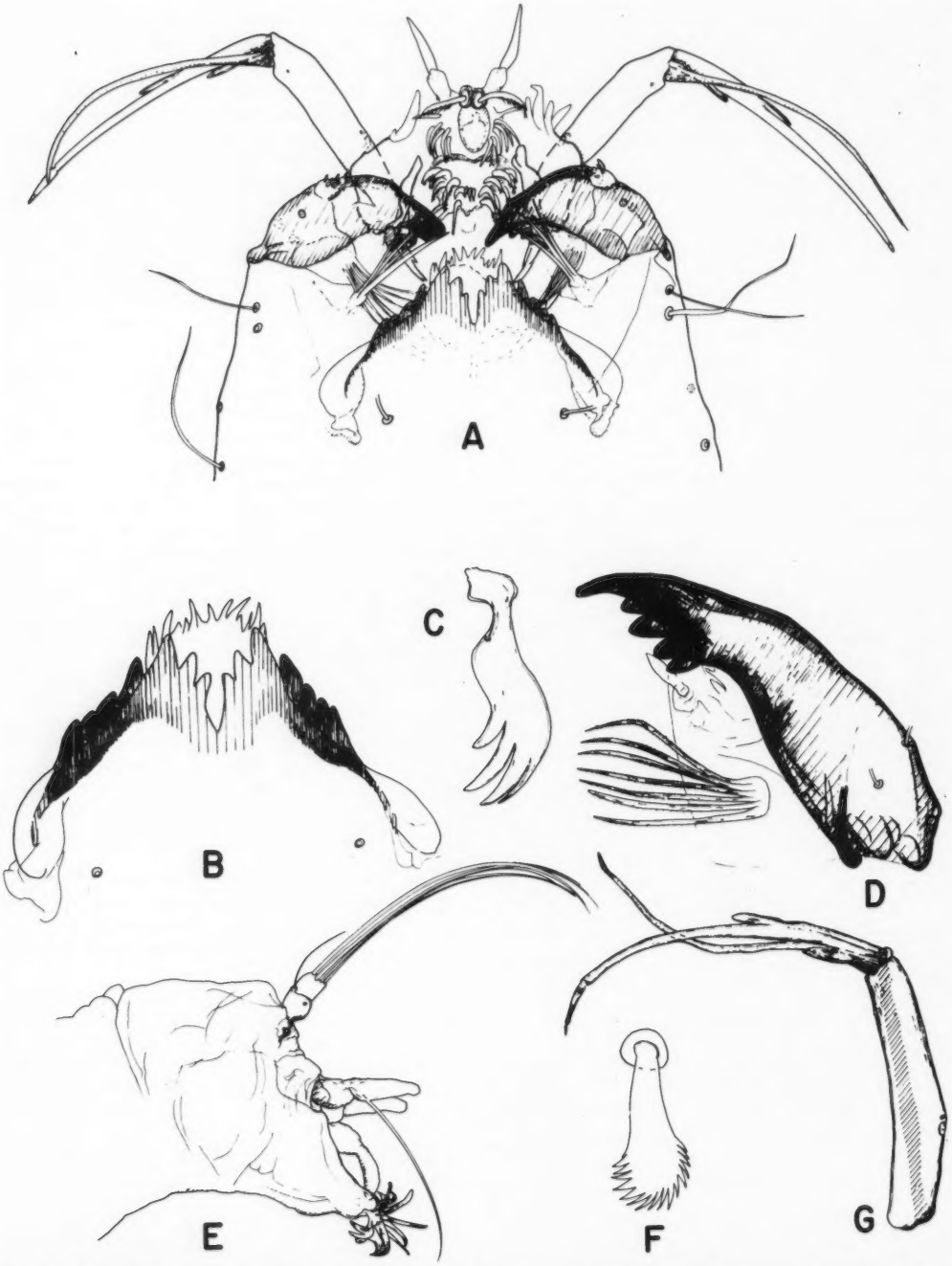

FIG. 3. *Heterotanytarsus perennis* n.sp., larva. A, head; B, mentum; C, premandible; D, mandible; E, caudal abdominal segments; F, setae anteriores (SI); G, antenna.

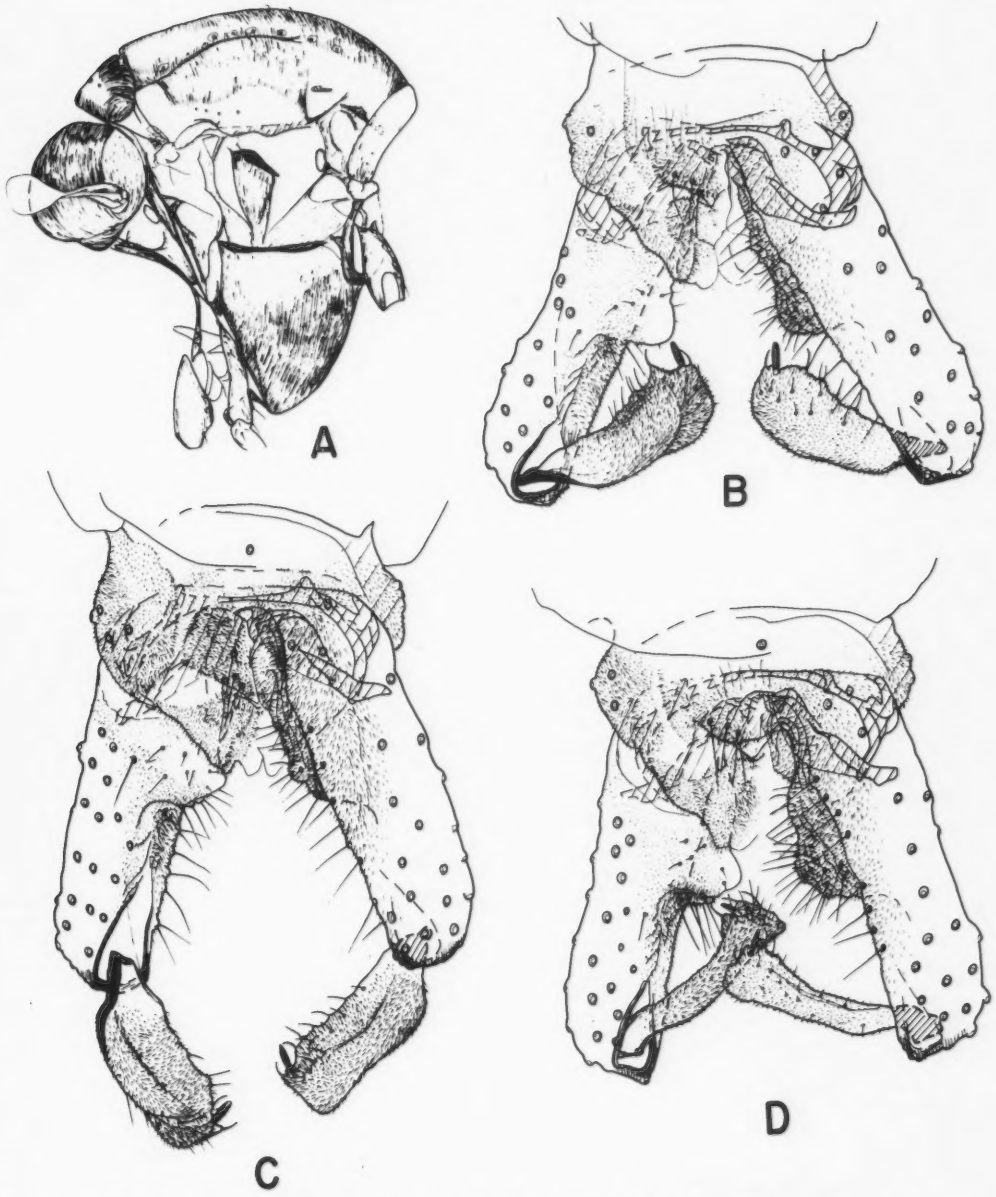

FIG. 4. *Heterotanytarsus nudalus* n.sp., male. A, thorax; B–D, hypopygium, variation.

Head — AR = 0.91–1.19, 1.04. Flagellomeres 1–3 with sensillae chaeticae. Outer verticals 2–4, 3; other temporals absent. Clypeus with 3–8, 5 setae. Cibarial pump, tentorium, and stipes as in Fig. 5B. Tentorium 119–154, 141 μ long; maximum width 22–27, 24 μ; width anterior of posterior tentorial pit 8–14, 12 μ; distance from apex to posterior tentorial pit 30–46, 39 μ. Stipes 90–111, 103 μ long. Palp lengths in microns: 19–24, 22; 31–41, 39; 63–78, 73; 57–80, 71; 86–114, 99.

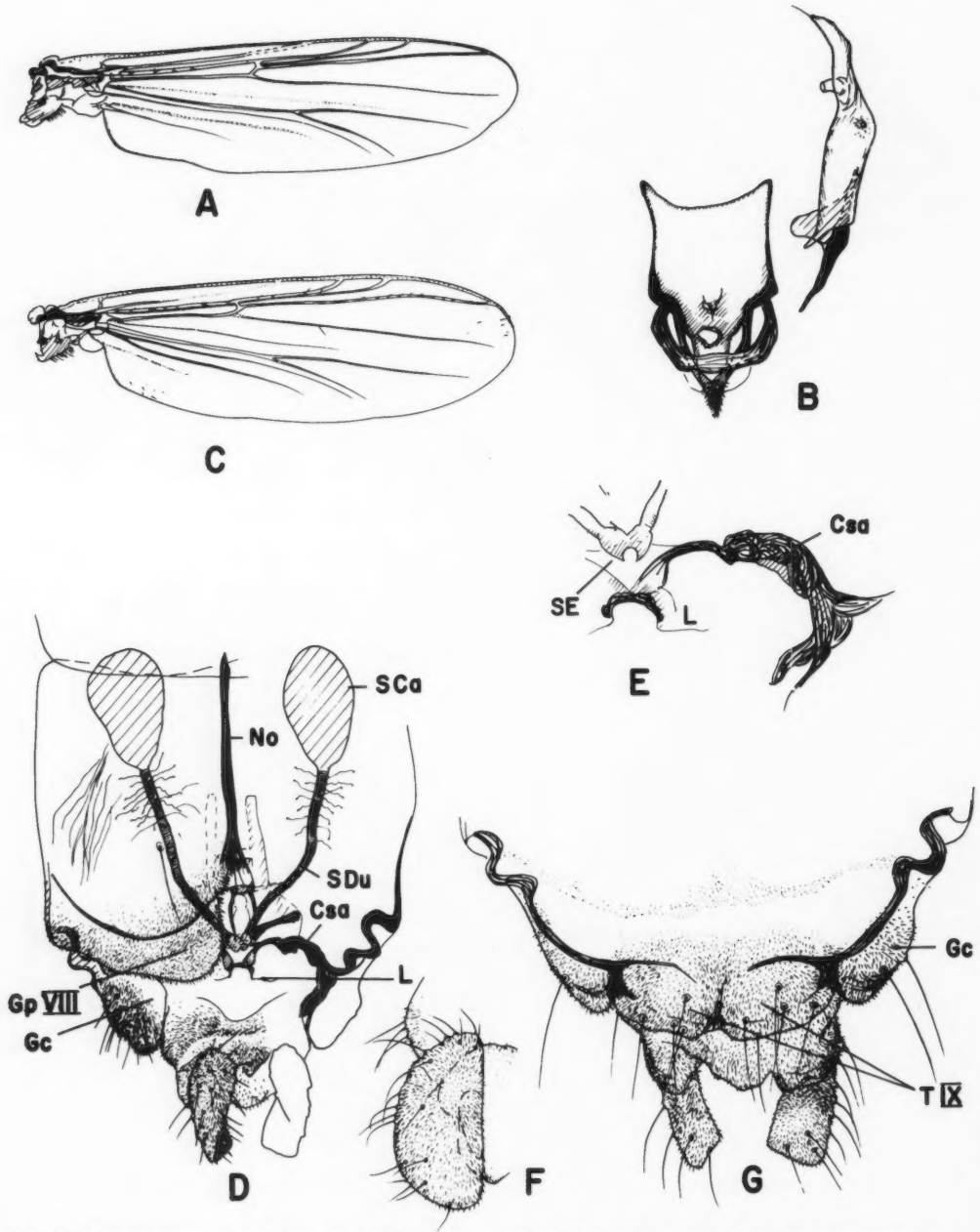

FIG. 5. *Heterotanytarsus nudalus* n.sp., A–B, male: A, wing; B, cibarial pump, labial lonchus, tormae, tentorium, and stipes (cross-hatched). C–G, female: C, wing; D, genitalia, ventral view; E, coxosternapodeme, spermathecal eminence, and labia; F, cercus, lateral view; G, genitalia, dorsal view. (Csa: coxosternapodeme; Gc: gonocoxite (IX); Gp. VIII: gonapophysis VIII; L: labium; No: notum; Sca: seminal capsule; SDu: spermathecal duct; SE: spermathecal eminence; T IX: tergite IX.).

Thorax (Fig. 4A) — Antepronotum with 3–6, 5 setae. Dorsocentrals 7–9, 8; acrostichals 2; prealars 4–6, 5. Scutellum with 4–5, 4 setae.

Wing (Fig. 5A) — VR = 1.26–1.42, 1.36. Extended part of costa 40–76, 51 μ long. Brachiolum with 1 setae; R with 3–7, 5; R_1 with 0–1, 0.1; R_{4+5} with 0–2, 0.2; extended part of costa with 0–4, 0.4 setae in addition to marginal setae. Squama with 10–17, 13 setae. Wing membrane without setae. Sensillae campaniformes 7–8 at base of brachiolum, 3–4 below setae, and 8–9 at apex of branchiolum, 1 on subcosta at humeral crossvein, 1 on Fr, and 1 about 80–190 μ from base on R_1.

Legs — Spur of front tibia 41–51, 46 μ long; spurs of middle tibia 16–23, 18 μ and 14–19, 16 μ long; of hind tibia 41–46, 44 μ and 12–20, 17 μ long. Width at apex of front tibia 24–35,30 μ; of middle tibia 27–38,32 μ; of hind tibia 30–43,39 μ. Tarsal pseudospurs absent. No true comb present, but spiniform setae apically on hind tibia end in an irregular apical row of 2–6 setae. Sensillae chaeticae present ⅓–½ from base on ta_1 of p_2 and p_3; 0–1, 0.2 on p_2; 1–2, 1.6 on p_3. Lengths (in microns) and proportions of legs:

	fe	ti	ta_1	ta_2
p_1	407–534,471	509–682,599	294–402,344	182–248,217
p_2	512–611,565	487–650,562	231–322,278	138–194,168
p_3	454–585,516	564–699,632	301–399,348	160–233,206

	ta_3	ta_4	ta_5
p_1	110–165,143	66–94,78	41–66,57
p_2	105–151,125	61–90,75	50–66,58
p_3	138–182,159	69–88,80	55–72,63

	LR	BV
p_1	0.54–0.59,0.57	2.75–3.28,2.87
p_2	0.46–0.51,0.49	3.17–3.44,3.30
p_3	0.54–0.57,0.55	2.76–3.15,2.95

	SV	BR (n = 8)
p_1	2.96–3.30,3.12	3.33–4.38,3.82
p_2	3.95–4.37,4.09	3.89–5.88,4.99
p_3	3.18–3.44,3.31	7.73–8.50,8.08

Hypopygium (Fig. 4B–D) — Ninth tergum with 4–10, 8 setae at base of the minute anal point; laterosternite IX with 3–6,5 setae. Phallapodeme 66–92, 84 μ long. Transverse sternapodeme 54–84, 72 μ long. Gonocoxite 196–244, 225 μ long; gonostylus 90–125, 112 μ long. HR = 1.90–2.18, 2.01; HV = 2.31–2.82, 2.51.

FEMALE IMAGO (N = 6, EXCEPT WHEN STATED OTHERWISE)

Length 2.62–2.80, 2.71 mm. Wing length 1.66–1.87, 1.74 mm. Total length/wing length: 1.45–1.68, 1.56. Wing length/profemur: 4.33–4.49, 4.42. Coloration blackish brown.

Head —Pedicel length/width (microns): 36–46, 41/58–65, 62. Flagellomeres length/width (microns): 68–76, 73/30–34, 31; 38–46, 42/24–30, 27; 42–49, 46/22–24, 23; 41–51, 45/22–26, 22; 88–108, 97/18–30, 23. AR = 0.45–0.58, 0.50. Outer verticals 2–3,3; other temporals absent. Clypeus with 4–9,6 setae. Tentorium 77–122, 100 μ long; maximum width 16–22, 18 μ; width anterior of posterior tentorial pit 7–11, 9 μ; distance from apex to posterior tentorial pit 14–19, 17 μ. Stipes 84–105, 95 μ long. Two vestigial ocelli present in 4 of 6 specimens 50–64, 57 μ (4) apart. Coronal suture complete. Palp lengths in microns: 18–27, 23; 34–38, 36; 57–62, 60 (5); 65–69, 68 (5); 88–100, 95.

Thorax — Antepronotum with 2–4, 3 setae. Dorsocentrals 8–9, 9; acrostichals 2; prealars 4–5, 5. Scutellum with 4–6, 5 setae.

Wing (Fig. 5C) — VR = 1.35–1.38, 1.37 (5). Extended part of costa 84–118, 92 μ (5) long. Brachiolum with 1 seta; R with 10–14, 12 (5); R_1 with 5–8, 6 (5); R_{4+5} with 11–14, 12 (5); M_{1+2} with 0–1, 0.4 (5); extended part of costa with 0–4, 2 (5) setae. Squama with 11–16, 13 setae. Cell r_{4+5} with 8–21, 14 (5) setae, m_{1+2} with 1–15, 6 (5); m_{3+4} with 2–4, 3 (5); an with 6–21, 12 (5) setae. Sensillae campaniformes as in male.

Legs — Spur of front tibia 22–28, 26 μ long; spurs of middle tibia 16–19, 18 μ (5) and 11–16, 13 μ (5) long; of hind tibia 42–46, 44 μ and 12–19, 15 μ (5) long. Width at apex of front tibia 31–36, 34 μ; of middle tibia 34–41, 37 μ; of hind tibia 41–47, 44 μ. Tarsal pseudospurs and hind tibial comb absent. Sensillae chaeticae present ⅓–½ from base on ta_1 of p_2 and p_3; 1–2, 2 (4) on p_2; 2–4, 3 on p_3. Lengths (in microns) and proportions of legs:

	fe	ti	ta_1
p_1	374–432,394	479–567,515	286–325,302
p_2	473–558,515	501–550,522	237–270,253
p_3	457–523,484	550–627,586	297–344,316

	ta_2	ta_3	ta_4
p_1	145–187,165	96–110.104	50–66,61
p_2	132–160,145	94–110,101	55–66,61
p_3	165–184,172	113–140,127	52–69,61

	ta_5	LR	BV
p_1	44–61,54	0.57–0.60,0.59	3.01–3.55,3.18
p_2	44–55,50	0.47–0.49,0.48	3.48–3.76,3.63
p_3	44–61,53	0.52–0.55,0.54	3.27–3.42,3.36

	SV	BR
p_1	2.94–3.08,3.01	2.50–3.30,2.93
p_2	3.96–4.18,4.10	2.86–3.89,2.92
p_3	3.33–3.45,3.39	4.78–6.36,5.60

Genitalia (Fig. 5D–G) — Gonapophysis VIII apparently with one simple lobe. Notum 111–143, 124 μ long. Gonocoxite with 8–13, 11 setae. Tergite IX faintly divided, with 7–12, 9 setae. Seminal capsules 73–100, 86 μ long; 47–55, 52 μ wide. Spermathecal ducts with common opening.

PUPA (N = 10)

Length 2.87–4.27, 3.42 mm. Length of thoracic horn/length of anal macrosetae 0.95–1.13, 1.03.

Cephalothorax — Thoracic horn (Fig. 2C) 298–374, 324 μ long; 80–110, 94 μ side; 2.82–4.13, 3.51 times as long as wide; apical spines conspicuously enlarged. Anterior precorneal setae (Fig. 2C) filamentous; 60–105, 82 μ long; located 7–20, 13 μ from median setae and 14–27, 21 μ from posterior setae. Median setae 40–92, 62 μ long; located 4–12, 8 μ from posterior setae. Posterior setae 155–200, 179 μ long; located 52–101, 75 μ from horn. Frontal plate (Fig. 2E) with rugulose warts and 69–140, 93 μ long frontal setae. Anterior dorsocentral setae (dc_1) 90–140, 124 μ long, located 18–54, 35 μ from dc_2; remaining dorsocentrals strong, but not spinelike; dc_2 60–80, 70 μ long, located 40–102, 71 μ from dc_3; dc_3 62–120, 93 μ long; located 33–70, 51 μ from dc; dc_4 60–90, 70 μ long.

Abdomen (Fig. 2B) — Shagreenation and chaetotaxy as shown in Fig. 2B with 4 filamentous setae on VII and VIII. Pedes spurii A present on IV–VIII; longest spinules 9–24, 18 μ long on IV; 12–28, 20 μ on V; 8–16, 14 μ on VI; 4–8, 5 μ on VII. Anal lobe with 13–22, 18 setae in fringe; 4–8, 6 of them mediad of the 294–330, 313 μ long anal macrosetae. Male genital sac with long fingerlike, apical projection.

LARVA — Unknown.

MATERIAL EXAMINED

HOLOTYPE: male, emergence trap above 10 m depth, Lake 122, Freshwater Institute Experimental Lake Area, Kenora, Ont., 17/5/68, S. S. Chang (CNC No. 13401). ALLOTYPE: female, same data as holotype. PARATYPES: 1 male reared from pupa, 23 males, 6 females, 1 mature male pupa, 27 pupal exuviae, emergence traps above about 10 m depth, Lake 122, Freshwater Institute Experimental Lake Area, Kenora, Ont., 17/5–14/6/68, 8/7/68 (1 male) and 18/9–16/9/68, S. S. Chang and A. P. Wiens.

Remarks

The male of this species differs from other known species of *Heterotanytarsus* by having an antennal ratio about 1 and no trace of setae on the wing membrane or on vein M_{1+2}. *Heterotanytarsus brundini* Fittk. (Fittkau 1956 p. 10), however, has only 1–2 setae either on M_{1+2} or on the wing membrane adjacent to the vein. *Heterotanytarsus nudalus* also has less numerous setae on the veins than the other known species and more numerous setae on the squama (10–17 compared to 3–7). The hypopygium closely resembles that of *H. apicalis* Kieff. (Brundin 1956a fig. 46).

The female differs from that of *H. brundini* (Fittkau 1956 p. 11) by having 8–13 setae on the gonocoxite compared to about 5 setae (Fittkau 1956 fig. 9). The thoracic horn with its enlarged apical spines apparently distinguish the pupae of this species from the other known pupae of the genus. *Heterotanytarsus nudalus* appears to be most closely related to *H. perennis* on the basis of the pupa.

Key to Males of Nearctic and Palaearctic Species of *Heterotanytarsus*

1 Gonostylus with well-developed outer projection; anal point broad, flat, lamellate, with numerous (about 30) setae at base (Fittkau 1956 fig. 3) ..... *H. brundini* Fittk. (Palaearctic)

Gonostylus at most with weak outer corner: anal point small to minute, triangular, with few (about 4–10) setae at base 2

2 AR about 1; wing membrane and M_{1+2} without setae; R_1 and R_{4+5} each with 2 or fewer setae; squama with 10–17 setae ..... *H. nudalus* n.sp. (Nearctic)

AR about 0.5–0.7; wing membrane and M_{1+2} with apical setae; R_1 with at least 3 setae, R_{4+5} with about 7–20 setae; squama with about 5–7 setae 3

3 Anal point small, but distinct (Fig. 1C); outer spur of hind tibia about 1/3 as long as inner spur; AR about 0.50–0.55 ..... *H. perennis* n.sp. (Nearctic)

Anal point minute, consisting of a small triangle (Brundin 1956a fig. 46); outer spur of hind tibia only 1/6–1/7 as long as inner spur; AR about 0.55–0.70 ..... *H. apicalis* Kieff. (Palaearctic)

Key to Pupae of Nearctic and Palaearctic Species of *Heterotanytarsus*

1 Thoracic horn cylindrical, slender, about 9 times as long as wide; segments VII and VIII each with 4 (occasionally 5) filamentous L-setae; three oral dorsocentrals located close together followed by a fourth at some distance ..... *H. brundini* Fittk. (Palaearctic)

Thoracic horn more or less flattened, about 2.8–6.6 times as long as wide; segment VII with 3, 4, or 5 filamentous L-setae; segment VIII with 4 or 5 filamentous L-setae; at most two oral dorsocentrals located close together 2

2 Segments VII and VIII each with 5 filamentous L-setae; spines on tergites reaching up to 15 μ in length ..... *H. apicalis* Kieff. (Palaearctic)

Segments VII and VIII each with 4 L-setae, 3 or 4 of them filamentous on VII; spines on tergites reaching up to 10–12 μ in length 3

3 Segment VII with 3 filamentous and 1 thinner L-setae; thoracic horn without conspicuously enlarged apical spines, 4.5–6.6 times as long as wide; anterior precorneal setae shorter than median precorneal setae and spinelike; three posterior dorsocentrals spinelike.. *H. perennis* n.sp. (Nearctic)

Segment VII with 4 filamentous L-setae; thoracic horn with conspicuously enlarged apical spines, 2.8–4.1 times as long as wide; anterior precorneal setae and three posterior dorsocentrals strong, but not spinelike ..... *H. nudalus* n.sp. (Nearctic)

Distribution and Ecology

Brundin (1949 p. 703) mentions that *H. apicalis* is a northern, oligotrophic species tolerant of varying degrees of humic substances. It is bivoltine with the first generation lasting from the beginning of April to the beginning of July in Southern Sweden, and from the middle of June to the middle of July in Northern Sweden, and with a second generation in September and October in Southern Sweden, and from the middle of August to the end of October in Northern Sweden. The prolonged spring emergence period is a result of the eurybathic nature of the species.

Heterotanytarsus brundini apparently is a rheophilous species as yet found only in Swedish Lappland (Fittkau 1956 p. 14).

Heterotanytarsus perennis as yet is known only from Marion Lake, B.C., a small oligotrophic forest lake (Hamilton 1965, p. 64). The larvae were common at all depths. The species is univoltine with an emergence period in May and early June.

Heterotanytarsus nudalus as yet is known only from Lake 122, Freshwater Institute Experimental Lake Area, Kenora, Ont., a typical oligotrophic lake. The species is bivoltine with an emergence period from the middle of May to the beginning of July, but with most of the emergence having taken place by the middle of June, and another emergence period from the middle of August to the middle of September.

The only other known Nearctic find is a larva from Sawlay Lake, Algonquin Park, Ont. This larva differs from that of *H. perennis* by having a narrower ventromental plate and basal antennal segment only 4.2 times as long as wide. It may possibly belong to *H. nudalus*.

Although *Heterotanytarsus* is known from only three Nearctic localities, these localities all conform to the kind of biotopes where *H. apicalis* could be expected to be found. Furthermore, the absence of the distinctive larvae from other investigated lakes indicates that the species is not present or at least not common in eutrophic and mesotrophic lakes and that it avoids the larger lakes. Most likely both species can be regarded as northern, oligotrophic members of chironomid communities.

Acknowledgments

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Behavioral Responses to Changes in Hydrostatic Pressure and Light During Larval Development of the Lobster *Homarus americanus*

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Larvae of the lobster *Homarus americanus* hatched and reared under laboratory conditions in shallow water responded to changes in hydrostatic pressure in a depth-regulatory manner. Responsiveness to instantaneous pressure increments ranging from 1 to 20 psi increased linearly up to 10 psi but varied little at higher pressures. Sensitivity to pressure changes was retained throughout larval life up to and including stage IV (megalopa) but stage III and IV larvae were much less responsive than stage I and II. Stage V (1st juvenile) lobsters were nonresponsive to pressure changes. Larvae were much less responsive (in terms of vertical movements) to changes in light intensity than to changes in pressure, and there were no clear, sustained phototactic or photopathic responses in the vertical plane.

In the sea, stage I, II, and III larvae showed limited downward movement when released at the surface and generally upward movement when released below the surface. Newly-molted stage IV larvae generally remained at the surface when released there and swam to the surface when released at the bottom; those 14–15 days after molting also tended to remain at the surface but when released at the bottom they generally remained there and found shelters. Newly-molted stage V juveniles generally remained where they were released, both at the surface and at the bottom, but those 13–15 days after molting when released at the surface generally swam to the bottom and found shelters, and when released at the bottom, remained there.

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Des larves du homard *Homarus americanus*, écloses et élevées au laboratoire en eau peu profonde, réagissent aux changements de pression hydrostatique en réglant la profondeur à laquelle elles se trouvent. La sensibilité à des augmentations subites de pression allant de 1 à 20 lb/po² augmente de façon linéaire jusqu'à une pression de 10 lb/po² mais change peu à des pressions plus fortes. Les larves conservent leur sensibilité aux changements de pression jusqu'au stade IV (mégalope) inclusivement, mais les larves aux stade III et IV réagissent beaucoup moins que celles aux stade I et II. Les homards au stade V (juvéniles 1) ne réagissent pas aux changements de pression. Les larves sont beaucoup moins sensibles (en termes de mouvements verticaux) aux changements d'intensité lumineuse qu'aux changements de pression, et il n'y a pas de phototaxie ni de photopathie définie et soutenue dans le plan vertical.

En mer, les larves aux stade I, II et III ont un mouvement restreint vers le bas lorsque relâchées à la surface, et généralement vers le haut lorsque relâchées sous la surface. Les larves au stade IV qui viennent de muer demeurent généralement à la surface lorsque libérées à cet endroit et nagent vers la surface lorsque libérées au fond; les larves qui ont mué 14–15 jours auparavant ont également tendance à demeurer à la surface, mais lorsque relâchées au fond elles y demeurent généralement et cherchent un abri. Les juvéniles au stade V demeurent en général là où ils ont été relâchés, tant à la surface qu'au fond, mais les individus qui ont mué 13–15 jours auparavant, lorsque relâchés à la surface, nagent généralement vers le fond et y trouvent un abri; lorsque relâchés au fond, ils y demeurent.

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PLANKTON sampling has shown that larvae of the lobster *Homarus americanus* are generally restricted to the surface layers (Templeman 1937; Templeman and Tibbo 1945; Scarratt 1973). Light seems to be the major factor controlling the vertical distribution of the larvae, but they apparently undergo a very limited diurnal vertical migration, the greatest depth from which they have been reported being 18 ft (5.5 m) (Smith 1937).

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Sensitivity to changes in hydrostatic pressure has been shown to be a common mechanism of depth-regulation (control of vertical distribution) in planktonic animals, including the larvae of decapod crustacea (Knight-Jones and Morgan 1966; Rice 1964, 1967; Sulkin 1973), and depth-regulatory responses to pressure changes by larvae of the European lobster, *Homarus gammarus*, were demonstrated by Ennis (1973).

Pressure sensitivity of this sort has not previously been demonstrated in *H. americanus* larvae. The purpose of this study was to determine if such a depth-regulatory response is present in this species and to investigate the possible importance of such a response in the control of vertical distribution.

Materials and Methods

The methods were essentially the same as those described by Ennis (1973) in a similar study on *H. gammarus* larvae. Larvae were obtained during the period June–August 1973 from ovigerous lobsters in holding tanks. Hatching usually occurred at night, shortly after darkness, and larvae were removed from the tanks soon after hatching and put in 68-liter polyethylene containers exposed to natural illumination. The water was changed and the larvae were fed *Artemia* nauplii daily. Despite cannibalism sufficient numbers of apparently healthy stage III larvae were obtained in these containers, but to get stages IV and V, stage III larvae were removed and held individually in 4.5-liter polyethylene containers in dim light.

The experimental column was a plexiglass tube 78 cm long, 6 cm internal diameter, with walls 4.5 mm thick. The column was pressure sealed at the top and was connected to a cannister fitted with an air pressure gauge. Pressure was increased in the cannister using an air pump, and pinchcock clamps were fitted so that pressure could be applied and released instantaneously.

In each experiment factors other than those under investigation, which might have influenced movements or distribution of larvae in the experimental column, were eliminated as far as possible. Only larvae of the same age and stage were used in an experiment, and all experiments were conducted at approximately the same time of day to eliminate the effects of any endogenous activity rhythm. Food organisms were not put into the column and relatively few larvae were used at a time to eliminate possible effects of high density. Clean water, at the same temperature and obtained at the same time as that in which the larvae were being reared, was used in the column. The experiments were of relatively short duration to eliminate the possibility of oxygen depletion and temperature layering in the column.

Experiments were carried out in darkness and in vertical overhead light of different intensities. The light source was a microscope lamp on top of the column giving a unidirectional parallel beam of bright white light from a 15-W bulb. Various light intensities were obtained by removing a 1.5-mm thick piece of frosted glass to give brighter light (very bright white light) or by placing one (bright green light), two (medium green light), or three (dim green light) pieces of translucent green polyethylene in front of the frosted glass to give light of progressively lower intensity. Relative differences in light intensity were recorded with a Sekonic Autolumi L-86 photo exposure meter with a Schott VG9 filter in an underwater housing. The exposure value readings for the various light intensities were within the range obtained to depths of 40 ft (12.2 m) in the sea during daytime.

Only healthy-looking, vigorous larvae were used and after each experimental run all were discarded. In the pressure response experiments 10 larvae were used in each run, except with stages IV and V where individuals were used. In the experiments on light response 20 larvae were used in each run, except for stage III when 10 larvae were used and stage IV where individuals were used. The result given for each experimental condition represents the average of three runs. Numbers of larvae in the top quarter of the column were counted before and after pressure changes and at intervals during periods of sustained pressure. In darkness counts were made quickly using a flashlight which was left on for about 3–4 s, but only those larvae already in the top quarter were exposed to this light because the bottom three-quarters of the column was wrapped in black plastic.

In the experiments on light response numbers of larvae in the top half of the column were counted to illustrate the response because the response to a change in light was much less vigorous and clear-cut than that to a change in pressure.

Pressure is given as pounds per square inch (psi) above atmospheric pressure. One atmosphere = 14.7 psi = 1000 millibars = 32.8 ft (10 m) of sea water.

Larvae were also released in the sea and observed by divers. These larvae were taken from the rearing containers about 30 min before the start of the dive and held individually in sealed 230-ml glass bottles of sea water. At the start of the dive light (exposure value) and temperature were recorded at 5- or 10-ft intervals (1.5 or 3.0 m) from the surface to 35 ft (10.7 m). Individual larvae were released at various depths and followed usually for 5 min but occasionally up to 15 min, and their depth at 1-min intervals after release was recorded. Unisuits were worn by the divers to maintain neutral buoyancy at all depths and avoid vigorous swimming movements near the larvae. The larvae could be observed from a distance of 3–4 ft (1.0–1.2 m) and exhaust bubbles from the scuba did not affect their vertical movements.

Results

Effect of Pressure Changes in Darkness

Stage I larvae from ½ to 3 days old were exposed to instantaneous pressure increases ranging

from 1 to 20 psi in darkness (Fig. 1). At pressures from 10 to 20 psi nearly all the larvae were swimming in the top quarter. Below 10 psi responsiveness to pressure increased approximately linearly with the magnitude of the pressure applied. There was no threshold of sensitivity within the range of pressures used and there was no clear change in responsiveness with age of larvae. At different rates of pressure (20 psi) application there was no significant difference ($P > 0.3$) in numbers in the top quarter (Fig. 2).

Stage I larvae remained responsive to pressure pulses (repeated pressure changes of the same magnitude) although there was a slight decline in numbers responding to later pulses (Fig. 3). The number of larvae responding to a second pulse (Table 1) was apparently not affected by the length of time between the pulses ($P > 0.7$) and the duration of the first pulse ($P > 0.2$).

Stage I larvae were subjected to a series of small pressure increases until 20 psi was reached, and

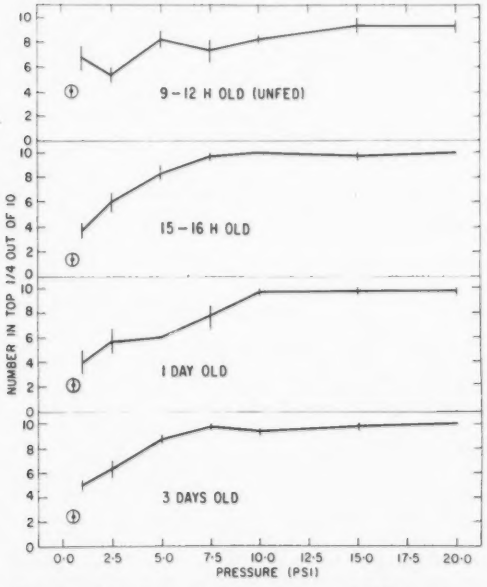

FIG. 1. Numbers of stage I larvae, of different ages, in top quarter of column after 5-min exposure to various pressures in darkness. Each pressure was applied instantaneously and the number at each pressure is the average of three runs using 10 fresh larvae which were discarded after each run. Circled points show average number of larvae in top quarter of column just before pressure applied in all runs. Vertical lines are ±1 standard error of the mean; for circled points the SE is equal to the diameter.

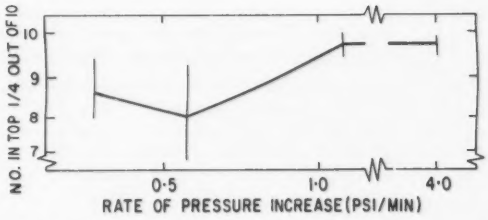

FIG. 2. Number of 9- to 12-h-old larvae in top quarter of column at 20 psi when pressure was increased at different rates in darkness. Vertical lines are ±1 SE.

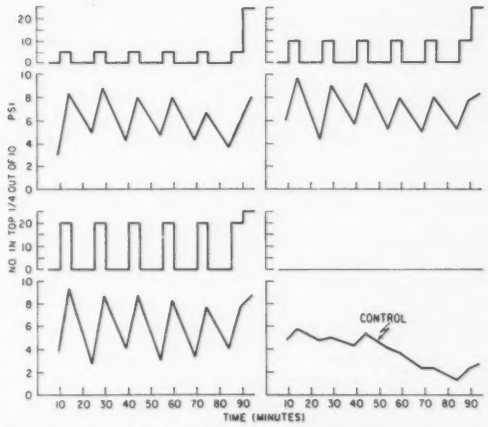

FIG. 3. Numbers of 9- to 12-h-old larvae in top quarter of column when pressures of different magnitude were pulsed in darkness.

then a series of small decreases (Fig. 4). The number of larvae in the top quarter of the column increased as the pressure rose to 15 psi but increases beyond this value had little effect. On lowering the pressure from 20 to 17.5 psi several larvae moved downwards, and at each subsequent decrease more larvae left the top of the column. At any given pressure the number of larvae in the top quarter was always less during the series of pressure decreases than it had been during the series of pressure increases.

EFFECT OF PRESSURE CHANGES IN OVERHEAD LIGHT

When experiments were conducted in overhead light, larvae of all stages responded immediately to an instantaneous increase in pressure, and all the larvae that were to move into the top quarter of the column had usually done so in less than

TABLE 1. Numbers of 9- to 12-h-old larvae in top quarter of column after 5 min at final pressure of 10 psi (a) after 10 min at 10 psi followed by 5, 15, or 25 min at 0 psi, (b) after 5, 15, or 25 min at 10 psi followed by 10 min at 0 psi.

a			b		
Time at 10 psi (*min*)	Time at 0 psi (*min*)	Numbers (Mean)	Time at 10 psi (*min*)	Time at 0 psi (*min*)	Numbers (Mean)
10	5	5, 7, 10 (7.3)	5	10	9, 10, 10 (9.7)
10	15	6, 8, 10 (8.0)	15	10	8, 9, 10 (9.0)
10	25	3, 10, 10 (7.7)	25	10	6, 10, 8 (8.0)

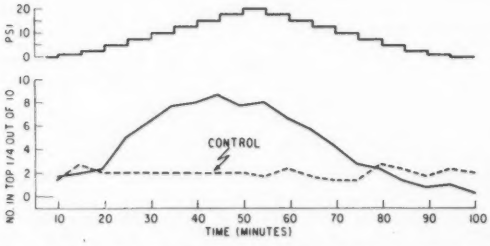

FIG. 4. Numbers of 9- to 12-h-old larvae in top quarter of column as pressure was increased and decreased slowly.

1 min. Upward movement was usually accomplished by increased beating rate of the swimming appendages (exopodites of thoracic appendages), but short bursts of rapid tail flexes were commonly used as well.

In bright green light stage I larvae were more responsive to small pressure increases than in darkness; in darkness smaller percentages of larvae responded to pressures of 5 psi and less ($0.1 > P > 0.05$ for young stage I and $P < 0.05$ for old stage I), but there was no difference in responsiveness to higher pressures (Fig. 5, cf. Fig. 1). Accommodation (downward movement in the column following the initial upward movement in response to the pressure increase) occurred during the short period of pressure exposure (5 min) but it was very slight at high pressures in stage I larvae. Stage II larvae were less responsive than stage I, particularly to pressures of up to 5 psi, and accommodated very rapidly even at high pressures.

Larvae of various ages were exposed to sustained pressures of 5 and 20 psi in darkness and in overhead light of various intensities (Fig. 6). No consistent pattern of variation related to light intensity was observed.

When pressures of 5 and 20 psi were sustained in overhead bright green light using larvae of

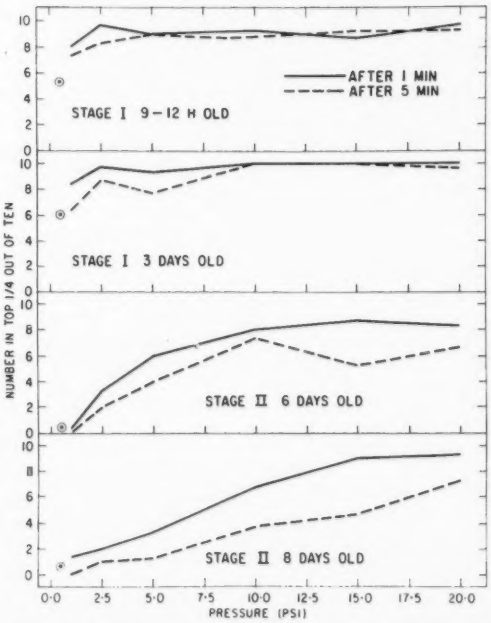
AFTER 1 MIN
AFTER 5 MIN
STAGE I 9–12 H OLD
STAGE I 3 DAYS OLD
STAGE II 6 DAYS OLD
STAGE II 8 DAYS OLD
NUMBER IN TOP 1/4 OUT OF TEN
PRESSURE (PSI)

FIG. 5. Numbers of stage I and II larvae, of different ages, in top quarter of column after 1- and 5-min exposure to various pressures in overhead bright green light. Larvae were left in column for 10 min before pressure applied. Each pressure was applied instantaneously and the number at each pressure is the average of three runs using 10 fresh larvae which were discarded after each run. Circled points show average number of larvae for all runs in top quarter of column just before pressure was applied.

different stages, accommodation was usually most rapid immediately following the initial response (Fig. 7). Stage III and IV larvae were much less responsive to pressure changes than earlier stages. Stage IV, the megalopa or postlarva, is the first stage with functional pleopods and it swims more

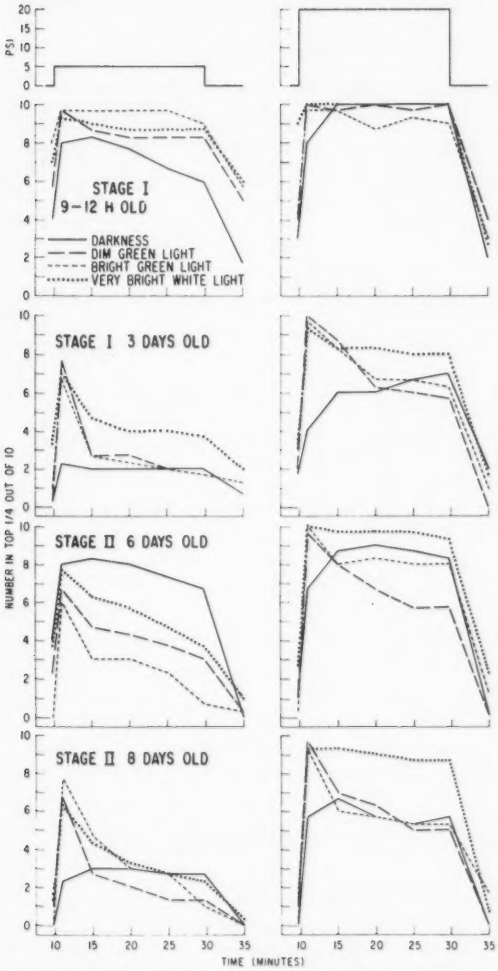

FIG. 6. Numbers of larvae of different ages in top quarter of column when pressures of 5 and 20 psi were sustained at different light intensities.

efficiently than the earlier (zoeal) stages. Specimens in this stage were generally very mobile in the experimental column, moving up and down before, during and after pressure exposure, but in spite of their activity, clear responses to pressure changes were recorded (Fig. 7). Juveniles in stage V are also very good swimmers and were equally active in the experimental column; however, their up and down movements did not appear to be associated with pressure changes (Fig. 7).

EFFECT OF SUDDEN CHANGES IN LIGHT INTENSITY

After 10 min in darkness the initial response of stage I larvae to overhead light was generally very slow upward swimming towards the light source (positive phototaxis) (Fig. 8). During exposure to constant light conditions there was slow downward movement away from the light source and the rate of downward movement was increased when larvae were returned to darkness. Within the range tested, light intensity appeared to have no effect on the initial response or on the rate of downward movement during the period of exposure. When light intensity was changed during a period of exposure to light, there was downward movement as the intensity decreased and usually upward movement or very slight downward movement as it increased.

Stage I larvae of different ages were more or less equally responsive to overhead light of the same intensity. Stage II larvae were only slightly less responsive, but stage III larvae were almost nonresponsive (Fig. 9). Stage IV larvae were observed individually and, although some were very mobile, their vertical movements did not appear to be in response to changes in light. The same pattern was seen at an additional 5 psi except that there were usually more larvae in the top half of the column throughout the experiments (Fig. 9).

OBSERVATIONS ON LARVAE RELEASED IN THE SEA

Stage I, II, and III larvae were released at the surface close to shore, and at depths of 10 (3.0 m), 20 (6.1 m), and at the bottom at 30 ft (9.1 m) (Table 2). When released at the surface more young stage I larvae remained there than moved down, but the opposite was true for the older stage I; chi-square (from 2×2 contingency table) shows that the results are barely significantly different ($P = 0.05$). For stages II and III numbers were equal. Chi-square tests indicate that for each age of stage I larvae the observations were not significantly different from those expected with the hypothesis that numbers moving down and remaining at the surface were equal ($P > .05$ for each). The average maximum downward movement during a 5-min period for all released at the surface was 1.8 ft (0.4 m) for young stage I larvae; for each of the other ages it was 4.5 ft (1.4 m). The maximum observed downward movement by individuals when released at the surface was 4 ft (1.2 m) for the younger stage I, 10 ft (3.0 m) for the older stage I, 14 ft (4.3 m) for stage II, and 23 ft (7.0 m) for stage

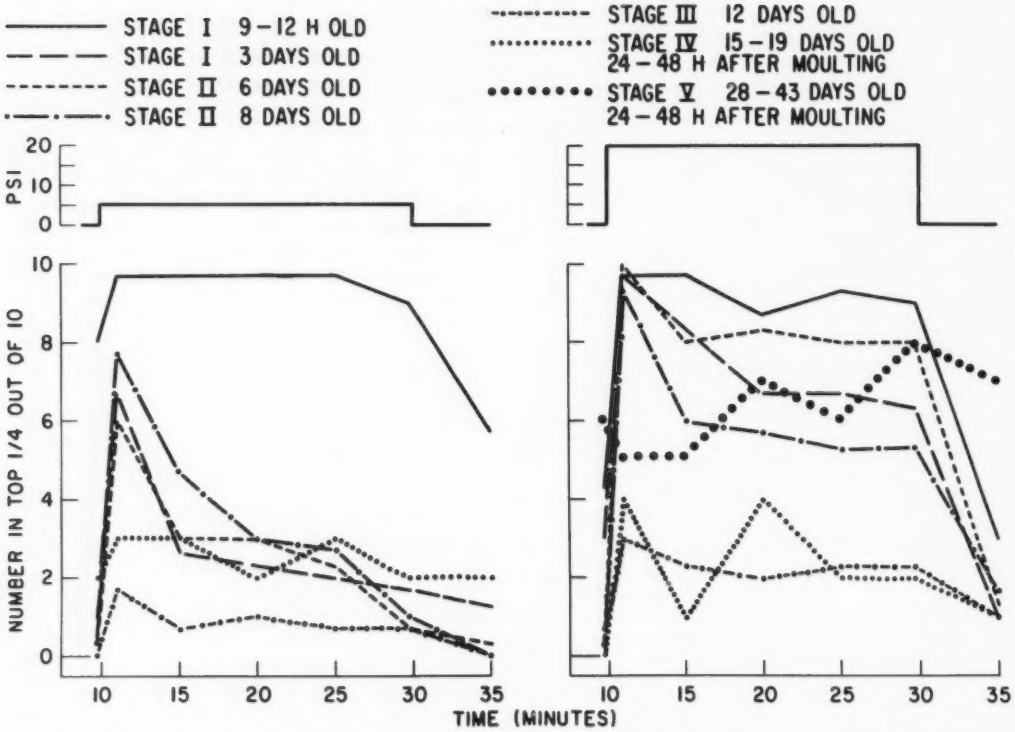

FIG. 7. Numbers of larvae of different ages in top quarter of column when pressures of 5 and 20 psi were sustained in overhead bright green light. (Lines for stages IV and V represent observations on 10 individual larvae.)

TABLE 2. Overall upward or downward movement of larvae of various ages, released at various depths, during 5 min from time of release.

Age (and stage) of larvae	Depth of release	Number released	Moved up	Moved down	No overall movement
16–18 h (stage I)	Surface	12	–	3	9
	10 ft (3.0 m)	11	10	1	–
	20 ft (6.1 m)	12	12	–	–
	30 ft (9.1 m)	11	11	–	–
3 days (stage I)	Surface	14	–	10	4
	10 ft	14	13	–	1
	20 ft	13	13	–	–
	30 ft	12	12	–	–
8 days (stage II)	Surface	11	–	6	5
	10 ft	10	6	1	3
	20 ft	11	8	–	3
	30 ft	12	12	–	–
11–14 days (stage III)	Surface	14	–	7	7
	10 ft	13	9	3	1
	20 ft	13	12	–	1
	30 ft	12	12	–	–

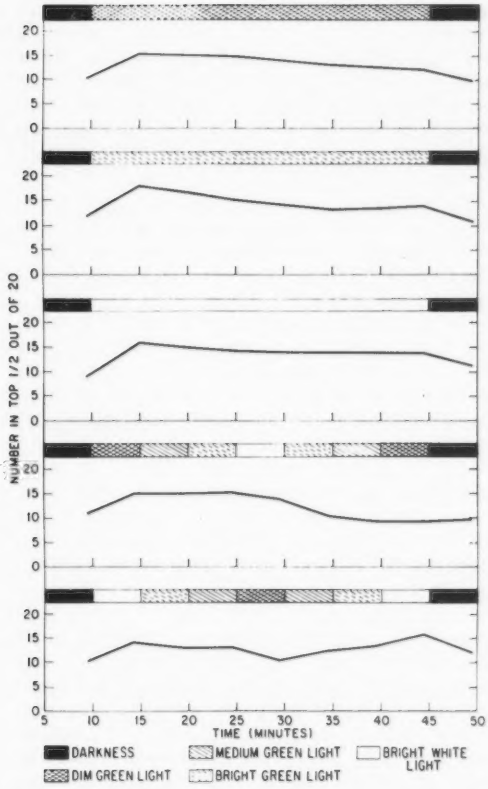

FIG. 8. Number of 9- to 12-h-old larvae in top half of column with sudden change in light intensity of different magnitude and with changing light intensity.

III. Often individuals moved downward some distance and then upward. Downward movement was by active swimming and not by passive sinking. It sometimes involved a slight change in orientation, especially during rapid downward movement, but more commonly it occurred without a change in orientation.

When released below the surface, larvae usually moved upward. However, individuals, particularly stages II and III, showed a tolerance for deep water by moving downward when released at 10 ft and by not moving upward when released at depths as great as 20 ft (Table 2). The amount of upward movement varied considerably among individuals; some swam all the way to the surface within 5 min, even when released at the greatest depth (30 ft), but others remained at intermediate depths and did not swim to the surface during periods of observation as long as 15 min. Occasionally individuals moved upward some distance and then downward. The average rate of upward movement increased with the depth of release and decreased over a 5-min period of observation for each depth of release (Fig. 10). The average rate of upward movement was generally somewhat greater for the younger stage I larvae but there was relatively little difference between the other ages, and variations were not consistent for each depth of release. The total upward movement after 5 min for 16- to 18-h-old stage I larvae released at 10 ft was significantly greater ($t = 2.27$; df = 21, $P < 0.05$) than that of older stage I but not of the others; for those released at 20 ft it was

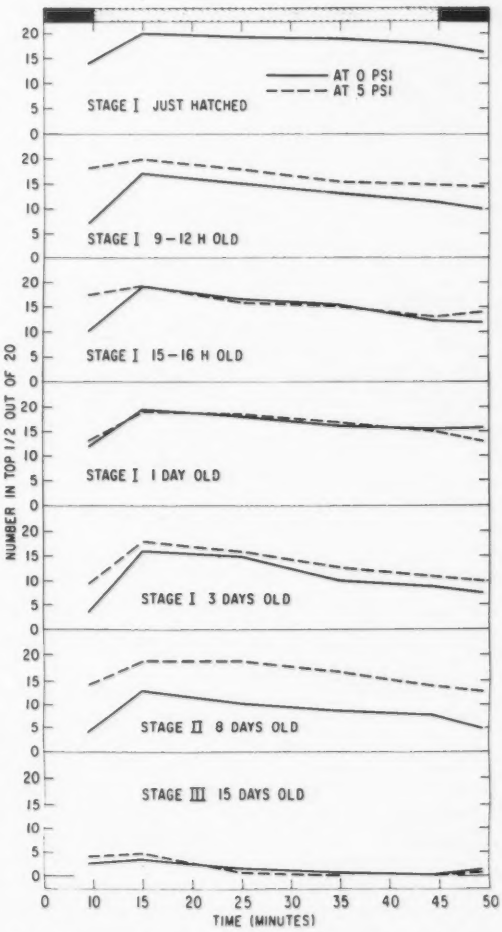
AT 0 PSI
AT 5 PSI
STAGE I JUST HATCHED
STAGE I 9–12 H OLD
STAGE I 15–16 H OLD
STAGE I 1 DAY OLD
STAGE I 3 DAYS OLD
STAGE II 8 DAYS OLD
STAGE III 15 DAYS OLD
NUMBER IN TOP 1/2 OUT OF 20
TIME (MINUTES)

FIG. 9. Numbers of larvae of different ages in top half of column during 35 min of bright green light, preceded and followed by darkness with pressure at 0 and at 5 psi.

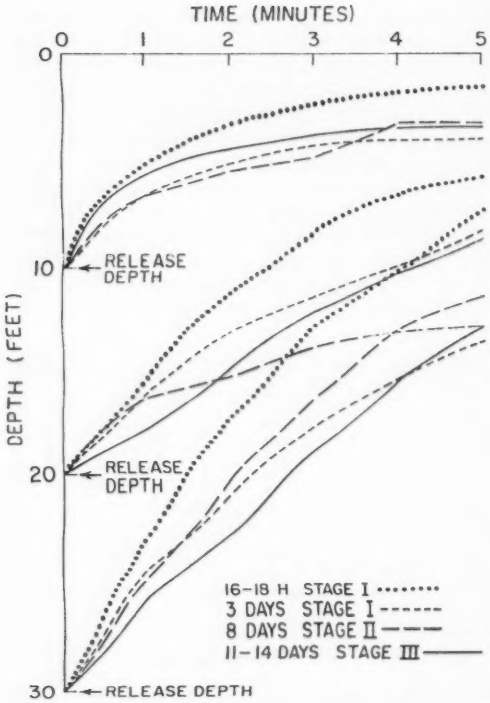

FIG. 10. Upward movement of larvae of different ages released in the sea at different depths. Each line represents an average of 6–13 observations. (Only larvae that moved up are included.) Lines were fitted to points (which are not shown) by eye and curved where the arrangement of points made this possible.

significantly greater than that of stage II larvae only ($t = 3.68$, df = 17, $P < 0.01$); and for those released at 30 ft it was significantly greater than that of older stage I and stage III ($t = 2.16$, df = 19, $P < 0.05$) but not stage II.

Larvae of each age were released on several different days when light conditions differed, and Fig. 10 records average movements under a range of light conditions. Average temperatures ranged between 12.7 and 13.9 C. Comparison of the movements of larvae of each age on the days of maximum and minimum average light readings indicates that downward movement by larvae released at the surface was greater and upward movement by larvae released at 10–30 ft (3.0–9.1 m) was less on brighter days (Table 3). Although some results on other days were inconsistent with this pattern, the data indicate the possibility of depth selection determined by light intensity.

Larvae of all stages were capable of fairly rapid vertical movements in both directions (Table 4) and 5 min appeared to be ample time for depth selection within the 30-ft depth range. In only one case, however, was there an indication of depth selection within relatively narrow depth limits by larvae of the same age released on the same day. In this case all the larvae released at various depths were within 4 ft of the surface after 5 min; in other cases the depth range was always much greater (Table 3).

Stage IV larvae and stage V juveniles, some less than 2 days after molting and others 13–15 days after, were released at the surface and on the bottom at 30 ft (9.1 m). Newly-molted stage IV larvae showed a very strong tendency to remain at the surface and to swim to the surface when released at the bottom (Table 5); those 14–15 days after molting behaved similarly when released at the surface, but when released at the bottom they tended to remain there and find shelters. The average age of these stage IV larvae 14–15 days after molting was 31 days; the average age of just-molted stage V was 43 days. This is a clear indication that bottom seeking and the characteristic shelter seeking behavior of post-larval lobsters develops before the end of stage IV. Newly-molted stage V juveniles similarly showed a strong tendency to remain at the surface but when released at the bottom they tended to remain and find shelters; however, most of those 13–15 days after molting that were released at the surface swam to the bottom while those released at the bottom remained there. The tendency for newly-molted stage V juveniles to remain at the surface instead of swimming to the bottom, as older stage V do, is not readily explained.

Discussion

The laboratory observations show clearly that depth-regulatory behavioral responses to changes in pressure are present in larvae of *H. americanus* and the larvae are more responsive, in terms of vertical movements, to changes in pressure than to changes in light.

The larvae were hatched and reared under laboratory conditions and it is uncertain to what extent their behavior can be considered typical of "natural" larvae. In addition, two factors make the experimental conditions different from those to which the larvae would be exposed in nature. The changes in pressure and light were instantaneous,

TABLE 3. Movements of larvae of various ages on days of different average light readings (figures in parentheses are number of larvae observed).

Age (and stage) of larvae	Date of release	Average depth (*ft*) of larvae after 5 min when released at				Depth range (*ft*) of individual larvae after 5 min	Average light reading
		Surface	10 ft	20 ft	30 ft		
16–18 h (stage I)	Aug. 16	2.8(3)	4.0(3)	6.3(3)	10.3(3)	1.5–19	10.2
	Aug. 30	1.3(3)	0.5(2)	11.0(3)	10.3(3)	0–17	9.3
	Aug. 28	0.5(3)	0.0(2)	3.5(3)	5.5(3)	0–9	9.1
	Aug. 31	0.3(3)	1.3(3)	2.3(3)	1.0(2)	0–4	9.1
3 days (stage I)	Aug. 17	7.2(3)	6.8(2)	16.0(2)	18.0(2)	3.5–20	10.1
	Aug. 27	3.7(3)	6.2(3)	14.0(3)	13.5(2)	3.0–15	9.1
	Sept. 3	2.0(3)	3.7(3)	2.5(2)	3.3(3)	1.0–20	8.3
	Aug. 23	4.2(3)	4.5(3)	3.0(3)	10.0(1)	0–10	7.8
8 days (stage II)	Aug. 20	5.8(3)	2.0(2)	11.7(3)	16.7(3)	0.5–19	9.7
	Aug. 22	2.0(2)	7.3(3)	12.0(2)	7.3(3)	0–14	9.7
	Sept. 5	2.5(3)	6.2(3)	19.0(3)	14.0(3)	0–26	9.3
	Aug. 21	4.2(3)	10.0(2)	17.5(2)	8.7(3)	1.5–20	8.4
11–14 days (stage III)	Aug. 25	5.8(3)	4.3(3)	10.3(3)	17.0(3)	1.5–23	9.9
	Aug. 24	7.8(3)	7.8(3)	5.0(2)	11.3(3)	0–19	9.8
	Aug. 29	0.0(3)	0.5(2)	9.7(3)	9.7(3)	0–19	7.2

TABLE 4. Maximum observed upward and downward movements during a 1-min interval by larvae of different ages released in the sea.

Age (and stage) of larvae	Maximum upward movement	Maximum downward movement
16–18 h (stage I)	11 ft (3.4 m)	4 ft (1.2 m)
3 days (stage I)	9 ft (2.7 m)	4 ft (1.2 m)
8 days (stage II)	8 ft (2.4 m)	6 ft (1.8 m)
11–14 days (stage III)	10 ft (3.0 m)	14 ft (4.3 m)
16–19 days (stage IV) less than 2 days after molting	12 ft (3.7 m)	8 ft (2.4 m)
28–30 days (stage IV) 14–15 days after molting	11 ft (3.4 m)	17 ft (5.2 m)
36–54 days (stage V) less than 2 days after molting	8 ft (2.4 m)	14 ft (4.3 m)
43–64 days (stage V) 13–15 days after molting	–	9 ft (2.7 m)

whereas in nature they would be gradual as the larvae move up or down in the water column; in the case of light there are also day/night changes. In addition the experimental apparatus did not permit fully compensatory vertical movements by the larvae in response to the changes. The results, however, can be considered in ecological terms. The absence of a clear threshold of pressure sensitivity, the accommodation of larvae even to high pressure, and the lack of clear, sustained phototactic and photopathic responses in the vertical plane indicate that they do not depth-regulate within well-defined limits. The observations indicate the ability to tolerate deep water, especially in older larvae. The fact that larvae remain responsive to pressure after a series of pressure changes (pulses) over a short period indicates that in nature they could undergo frequent changes in

TABLE 5. Movements of lobsters in stages IV and V when released at the surface and at the bottom in 30 ft (9.1 m).

Age (*days*)	Stage	Time since molting (*days*)	Level of release	Number released	Remained where released	Moved to surface from bottom or to bottom from surface
16–18	IV	0–2	surface	9	7	0
16–18	IV	0–2	bottom	12	2	10
28–31	IV	14–15	surface	6	4	1
28–31	IV	14–15	bottom	11	9	2
35–54	V	0–2	surface	9	8	1
35–54	V	0–2	bottom	8	7	1
43–64	V	13–15	surface	5	1	4
43–64	V	13–15	bottom	2	2	0

vertical position without losing their sensitivity and responsiveness to pressure changes. The stimulation for upward swimming increased with the magnitude of the depth increase. However, following a series of slight pressure increases to a high level, slight decreases resulted in considerable downward movement, suggesting that in nature if larvae undergo an extensive downward movement, a slight upward movement could reduce the pressure stimulus to a point where further upward swimming need not occur.

The observations on larvae released in the sea indicate that larvae were stimulated to greater upward movement and less downward movement on dull than on bright days, suggesting the possibility of some degree of depth selection determined by light intensity. When released in deep water they were stimulated to more rapid upward movement than when released in shallower water. The greater stimulation could have resulted from the increased pressure or decreased light intensity or a combination of the two. The decrease in the rate of upward movement with time for all depths of release probably resulted from the decrease in stimulation for upward swimming as larvae moved to shallower water. As with the laboratory experiments there was no clear indication that lobster larvae depth-regulate within well-defined limits and, instead of concentrating at or near the surface, many larvae, especially older ones, showed a tolerance for deeper water. The results of this study are generally inconsistent with the light controlled, short distance, daily vertical movements of *H. americanus* larvae suggested by Templeman and Tibbo (1945). They also indicate that estimates of the survival of lobster larvae based on the abundance of the four successive stages in the surface plankton (Scarratt 1964; Lund and Stewart 1970) may be inaccurate. Scarratt (1973) presented evidence which suggested that settlement of *H. americanus* larvae to the bottom takes place before the end of stage IV and that estimates of larval survival based on abundance of stage IV larvae in the surface plankton are therefore low. Observations presented in this paper on the behavior of older stage IV larvae released in the sea support this idea.

Larvae of *H. gammarus* had previously been observed in laboratory experiments similar to those described here for the American species (Ennis 1973). Comparison of the results in most cases indicates only slight differences in responsiveness to changes in pressure and light between the species. The differences observed do not explain why *H. gammarus* larvae appear to be virtually absent from the plankton whereas *H. americanus* larvae are found abundantly in the surface plankton during the larval period.

The apparent difference in vertical distribution of the larvae of the two species may be an artifact caused by relative differences in abundance or survival. Even in Canadian waters in the Bay of Fundy larval lobsters are rarely taken in the plankton despite the presence of an abundant adult population in the area (Huntsman 1923), apparently because of very poor survival due to

low temperatures. It is unreasonable to assume, however, that unfavorable conditions for larval survival exist throughout the range of *H. gammarus*, and the reason for their apparent absence from the plankton is unclear.

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Spawning of Lake Whitefish, *Coregonus clupeaformis*, and Round Whitefish, *Prosopium cylindraceum*, in Aishihik Lake and East Aishihik River, Yukon Territory

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BRYAN, J. E., AND D. A. KATO. 1975. Spawning of lake whitefish, *Coregonus clupeaformis*, and round whitefish, *Prosopium cylindraceum*, in Aishihik Lake and East Aishihik River, Yukon Territory. J. Fish. Res. Board Can. 32: 283–288.

Spawning grounds used by lake (*Coregonus clupeaformis*) and round whitefish (*Prosopium cylindraceum*) were discovered in the course of an investigation on effects of hydroelectric development. The spawning period for lake whitefish extended from early November to at least mid-December 1973. Lake whitefish spawned over silt and *Potomogeton* in water which had little current and was 2.0–2.5 m deep. Spawning of round whitefish was probably completed in November. Round whitefish spawned during the day. Eggs were apparently broadcast over a variety of substrata ranging from silt and *Potamogeton* to gravel and boulder. Round whitefish eggs were deposited in both fast and slow current at depths ranging from 0.7 to 2.5 m. Although deposited in a range of habitats, round whitefish eggs seemed to be most abundant on gravel in fast current at depths less than 1 m.

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On a découvert des frayères utilisées par le grand corégone (*Coregonus clupeaformis*) et le ménomini rond (*Prosopium cylindraceum*) au cours d'une étude sur les effets d'un développement hydro-électrique. La période de fraie du grand corégone a duré du début de novembre à au moins la mi-décembre 1973. Le grand corégone a frayé au-dessus de fonds de vase et de *Potamogeton*, dans une eau de faible courant et de profondeur de 2.0–2.5 m. La fraie du ménomini rond s'est probablement terminée en novembre. Le ménomini rond a frayé de jour. Les oeufs furent apparemment dispersés sur une variété de substrats, allant de vase et *Potamogeton* à gravier et galet. Les oeufs du ménomini rond ont été déposés à la fois dans un courant rapide et un courant lent, à des profondeurs variant entre 0.7 et 2.5 m. Quoique déposés dans divers habitats, les oeufs du ménomini rond ont semblé plus abondants sur le gravier, dans un courant rapide et à des profondeurs de moins de 1 m.

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SPAWNING round whitefish, *Prosopium cylindraceum*, were discovered during an impact study of hydroelectric development. Finding gravid round whitefish and drifting eggs prompted us to attempt further observations on drifting eggs, spawning behavior, and egg deposition. While sampling for round whitefish, we captured gravid lake whitefish, *Coregonus clupeaformis*. Eggs of both species were recovered from the substratum.

For both lake and round whitefish, spawning habitats have been described by other workers (Hart 1930; Normandeau 1969; and Bidgood 1973). However, some of the habitats where spawning has been reported previously differ from habitats where eggs were deposited in this study. It is important to have good descriptions of spawning habitat so that potential spawning areas can be recognized and protected against damage resulting from projects which produce major environmental changes.

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Methods — Aishihik Lake is located roughly 160 km northwest of Whitehorse (Fig. 1). The lake has a surface area of approximately 150 km². Ranked from most to least abundant, the fish species composition of the lake is: lake whitefish, lake trout, Arctic grayling, round whitefish, burbot, northern pike, and longnose sucker (Kussat 1973). The same species plus slimy sculpin and rainbow trout have been observed in the East Aishihik River system which flows out of Aishihik Lake (Walker 1972). The volume of water discharged by the East Aishihik River ranges from 35 to 250 m³/s.

Drifting eggs were collected in the locations shown in Fig. 1. A fyke net designed for catching fish was used for catching eggs at location DN 1. The portion of the net which retained eggs was a cylinder (diameter 46 cm) with round mesh (diameter 1.6 mm). The other driftnets were rectangular with round mesh (diameter < 1 mm). At DN 2, the net measured 53 cm horizontally by 43 cm vertically; two nets were set at DN 3, each measuring 61 cm horizontally by 32 cm vertically. All of the driftnets rested on the substratum. Fish were captured in gillnets set in several locations near the outlet of Aishihik Lake (Fig. 1).

Current velocity was measured by timing a stick floating for a measured distance. Surface to bottom temperatures were measured with a thermister (Yellow Springs Instrument), whereas surface measurements were made with a hand thermometer.

Eggs were collected from the substratum with a round dipnet (20 cm diameter with round mesh < 1 mm diameter). The usual method of egg collection was to bounce the net slowly over the surface of the substratum against the current. This process was repeated 5 times at each site, and all of the eggs and substratum obtained were treated as one sample. At a few sites, we collected eggs by pressing the net firmly against the bottom and disturbing the top few centimeters of substratum for an area of about 600 cm² upstream of the net. Eggs were placed in a dish of water, and their diameters were measured with Helios dial calipers. Each egg was held firmly by the jaws of the calipers, but the pressure was adjusted so that the egg would slip through the jaws upon breaking the surface tension of the water.

Results — Lake and round whitefish were the predominant species captured in the gillnets. For both lake and round whitefish, spawning presumably occurred from the first to the third week of November, as gravid and spawned-out fish were captured at these times. The spawning period for lake whitefish extended to mid-December since a ripe female was captured December 12. Only 1 of 13 round whitefish captured in December was mature, and it was a spawned-out male; so round whitefish spawning was probably completed in November. Most lake whitefish were caught at night whereas most round whitefish were caught during the day.

Many of the fish captured had tubercles which were small, hard projections on the scales of the fish. These tubercles were particularly prominent along the lateral line, but some fish had them on their nape as well. Tubercles were present only on mature individuals. They were more often found on male (74%) than on female (17%) round whitefish. Tubercles occurred with nearly equal frequency on male and female lake whitefish.

Spawned-out fish were examined for scars which might have resulted from digging redds. Most fish had gillnet scars, but none had marks or extensive scale loss on the ventral surface.

Drifting eggs were captured much more frequently during the day than at night (Table 1). On November 21 and 22, the round whitefish spawning was interrupted by gillnetting and egg collecting on the spawning grounds, and this presumably accounts for the reduction in number of drifting eggs. The low catch of eggs in December suggests that the spawning was complete by that time. The same diurnal pattern of an increase in drifting round whitefish eggs during the day was observed in a driftnet set farther downstream (Fig. 1, DN 2). Few eggs, however, were captured in this net (six eggs per 24 h). Two driftnets set below Canyon Pond (Fig. 1, DN 3) were checked for eggs once each day, and the combined catch for the two nets over the 48-h period November 15–17 was 273 round whitefish eggs.

Egg collection was undertaken in a grid upstream of the fyke net (Fig. 2). Table 2 shows the number of eggs collected and describes each site. Unpreserved lake whitefish eggs ranged from 2.36 to 2.60 mm in diameter and round whitefish eggs from 3.13 to 3.44 mm. These sizes are similar to those observed by Booke (1970). After preservation in 10% formalin, the diameter of eggs in a lake whitefish ovary ranged from 1.50 to 2.15 mm, whereas eggs from two round whitefish ovaries ranged from 2.20 to 2.67 mm. In neither species did the eggs adhere strongly to each other, to vegetation, or to rocks.

Current speeds and temperatures were observed for the grid (Fig. 2) on November 22, 1973. The surface current speed increased with the grid numbers from a low but unmeasured value at locations 1 and 2. Between locations 4 and 5, the surface current speed was 31 cm/s; between locations 5 and 7, the speed was 63 cm/s. When the air temperature was approximately–23 C, the following temperature profile was observed at location 1 of the grid:

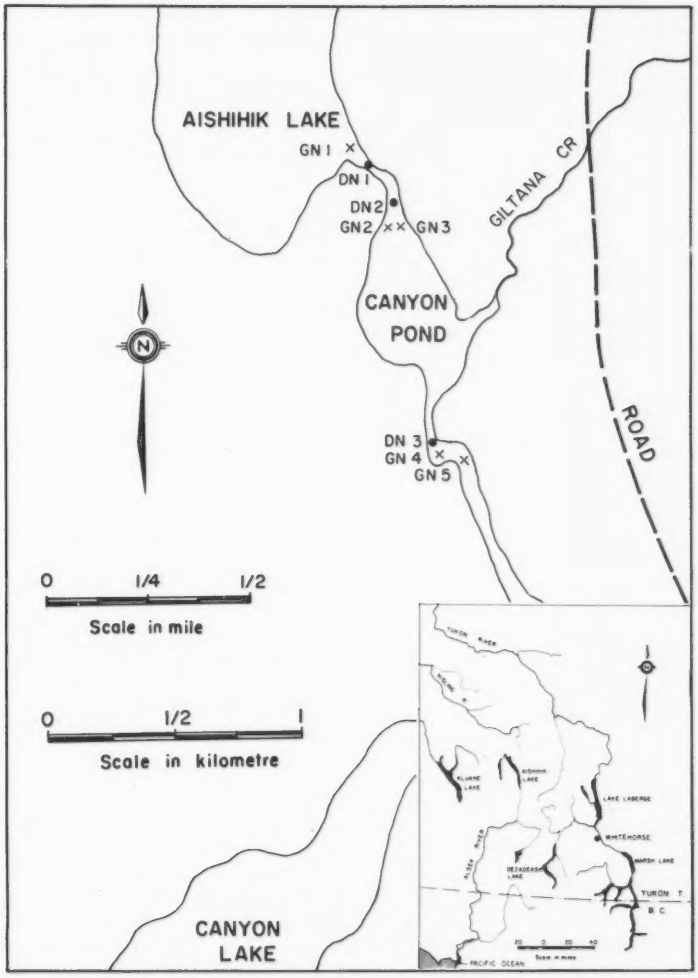

FIG. 1. Location of gillnet (GN) and driftnet (DN) sites in Aishihik Lake and the upper East Aishihik River.

Depth (*m*)	Temp (*C*)
0.0	0.8
0.5	1.4
1.0	1.7
2.0	1.7
2.5	2.2

From November 7 to 23, the surface water temperature at location 10 ranged from 1.4 to 1.6 C.

Eggs of lake whitefish were collected mainly over silty substratum in deep water whereas round whitefish eggs were collected at most locations on the grid (Fig. 2, Table 2). Although the difference in method makes direct comparison tenuous, round whitefish eggs seemed to be densest over gravel in fast water. Agitation of a small area upstream of the net usually released about as many eggs as were obtained from larger areas in other locations. On December 12, locations near 7C, 8, and 9 were again sampled for eggs by disturbing the upstream gravel. The egg density was much

TABLE 1. Number of drifting round whitefish (*Prosopium cylindraceum*) eggs collected in the fyke net set in the outlet of Aishihik Lake in 1973. Dashes indicate that the net had just been reset at the date and time specified. Sunrise was about 0830 and sunset about 1630.

Date	Time	Number eggs/h
Nov. 8	1730	–
Nov. 9	1100	4.92
Nov. 15	1100	–
Nov. 15	1700	156.17
Nov. 16	0900	2.00
Nov. 16	1215	25.32
Nov. 16	1730	35.00
Nov. 17	0845	1.91
Nov. 17	1430	26.55
Nov. 19	1700	19.58
Nov. 20	0930	0.91
Nov. 20	1300	–
Nov. 20	1500	35.00
Nov. 20	1700	18.00
Nov. 21	0900	2.94
Nov. 21	1700	4.00
Nov. 22	0900	2.25
Nov. 22	1730	7.42
Nov. 23	0930	1.44
Dec. 10	1700	–
Dec. 11	1300	0.20
Dec. 11	1700	0.00
Dec. 12	1100	0.06
Dec. 12	1615	0.00
Dec. 13	1130	0.00

lower as only five round whitefish eggs were obtained by disturbing approximately 1.5 m^2 of gravel.

Some observations were made on round whitefish swimming above the egg sampling grid. All observations were made between 1100 and 1500 PST, and round whitefish was the only species seen. The round whitefish showed no site attachment but moved slowly over a wide area. Groups of round whitefish usually moved in the same direction together. Even though individuals often came into close contact by moving above or below other individuals, no agonistic behavior was observed. Contrary to observations of Normandeau (1969), individuals were not paired. The round whitefish usually remained within a few centimeters of the bottom, but they did not disturb the substratum by digging. They frequently surfaced, breaking water with their backs and returning to positions near the bottom. No releases of eggs and milt were observed. One round whitefish performed a lateral display by turning on its side and vibrating near another individual.

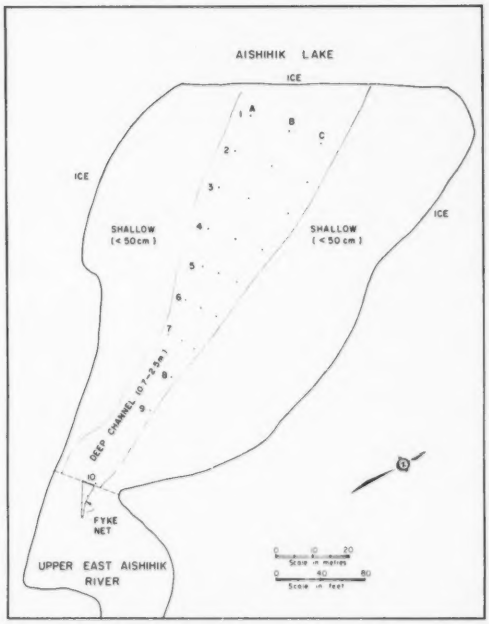

FIG. 2. The egg sampling grid near the outlet of Aishihik Lake. The fyke net is located at position DN 1.

Discussion — Lake whitefish deposited their eggs over silt and *Potamogeton* where there was slight current. Other workers report that lake whitefish spawn over boulders (Hart 1930) and in streams (Lindsey 1963). Although we found few eggs in areas with boulder substratum, eggs may have been lodged in crevices where they would have been invulnerable to collection with the net. Hart (1930) collected lake whitefish eggs from crevices between boulders. Bidgood (1973) found that lake whitefish spawned over sand and gravel as well as boulders in Alberta. Indeed, some spawning was observed in shallow water (< 1 m) over a sand-silt substratum (Bidgood 1972). We found few lake whitefish eggs over sand or gravel or in swift current. Thus lake whitefish in different locations apparently deposit eggs in different habitats.

The spawning season of lake whitefish in Aishihik Lake extended from the beginning of November to at least mid-December. This duration is longer than in some areas (Hart 1930; Qadri 1968) but comparable to that reported for other regions (Van Oosten and Hile 1949; Bidgood 1972). Lake whitefish were caught on the spawning ground at night and were not ob-

TABLE 2. Number of eggs collected from various substratum types and depths. The sampling locations are given in Fig. 2. Substratum composition indicates the percentage of bottom area which is covered by substratum of each type.

	Substratum composition (%)									
Sample no.	Silt	Sand (0–2 mm)	Gravel (2–50 mm)	Gravel (50–250 mm)	Boulder (25–1000 cm)	*Potamogeton* present	Periphyton present	Depth (*m*)	No. eggs lake whitefish	No. eggs round whitefish
1A	100	0	0	0	0	×		2.5	11	19
*1B	100	0	0	0	0	×		2.8	0	0
**1B	100	0	0	0	0	×		2.8	23	4
1C	0	0	0	0	100		×	2.0	0	0
**2A	100	0	0	0	0	×		2.5	19	2
*2A	100	0	0	0	0	×		2.5	55	23
2A	70	0	0	0	30		×	2.5	0	1
2A	70	0	0	0	30		×	2.5	0	0
2B	100	0	0	0	0	×		2.5	20	9
2B	100	0	0	0	0	×		2.5	4	0
2B	100	0	0	0	0	×		2.5	12	2
2B	100	0	0	0	0	×		2.5	34	15
**2B	100	0	0	0	0	×		2.5	13	0
2B	100	0	0	0	0	×		2.5	37	1
2C	0	0	40	40	20			2.5	3	1
3A	0	80	0	20	0		×	2.0	0	0
3B	0	0	60	20	20		×	2.0	0	0
3B	0	0	60	20	20		×	2.0	4	1
3C	0	10	60	20	10		×	2.0	6	1
4A	0	60	0	40	0		×	2.0	0	8
4A	0	0	0	20	80		×	2.0	0	0
4B	0	20	0	0	80		×	2.0	0	0
4C	50	0	0	20	30		×	2.0	0	0
5A	0	20	0	10	70		×	2.0	0	1
5B	0	0	0	20	80		×	1.5	0	0
5C	0	20	20	0	60		×	1.5	0	0
6A	0	0	0	40	60		×	1.0	0	1
6B	0	0	0	35	65		×	1.5	0	0
6C	0	0	30	20	50		×	1.0	0	0
7A	0	0	0	20	80		×	1.0	0	2
7B	0	0	0	100	0		×	1.2	0	0
7C	0	0	0	20	80		×	1.0	0	0
†7C	0	0	100	0	0		×	0.7	0	8
†7C	0	0	100	0	0		×	0.7	0	23
†8	0	0	100	0	0		×	0.7	0	9
†9	0	0	0	100	0		×	0.7	0	13
†10	0	0	100	0	0		×	1.0	0	1

*Sampled mud.
**Sampled surface of vegetation.
†Sampled by disturbing gravel upstream of net.

served there during the day. This is consistent with other observations that lake whitefish spawn at night (Hart 1930; Qadri 1968; Bidgood 1972).

The round whitefish deposited eggs throughout the sampling grid which represented substratum ranging from mud to gravel and boulders and current speeds from slow to fast. The eggs seemed to be most densely deposited in areas of gravel and swift current. In such areas, bouncing the net over the substratum did not sample as effectively as agitating the substratum upstream of the net. As described previously, the net was probably ineffective in capturing eggs deposited over boulder substratum. Drifting eggs were captured

both downstream of Aishihik Lake and also downstream of Canyon Pond. In more northerly regions of Yukon Territory, round whitefish probably also spawn over gravel areas of streams since fry have been captured in such locations shortly after spring breakup (Bryan 1973). Previously, round whitefish have been observed spawning in still water over gravel and rubble at depths ranging from 15 cm to 14 m (Normandeau 1969). Observations of fishermen suggest that spawning in the Great Lakes occurs over gravel and boulders in still water (4–12 m deep) and at the mouths of rivers (Koelz 1929).

The spawning season for round whitefish at Aishihik Lake probably extended from the beginning to the end of November. The absence of adult fish from locations occupied during spawning and the reduced drift of eggs indicated that spawning was complete by December. Spawning of round whitefish at Nueltin Lake, N.W.T. (Harper 1948) probably occurred in October and November. In Lake Michigan, most round whitefish had spawned by mid-December (Mraz 1964). In the Pyasina and Anadyr' rivers, spawning occurs in October and November (Berg 1948). In northern Yukon Territory, round whitefish may spawn in late August (Bryan 1973). Gravid round whitefish with tubercles were caught in the Driftwood River, and grayling from the same area had eaten fish eggs. The reduced drift of round whitefish eggs at night combined with the reduction of numbers on the spawning ground at night indicates that spawning occurred during the day. Most other whitefish species spawn at night (Fabricius and Lindroth 1954; Alt 1969; Hart 1930).

Round whitefish apparently spawned by broadcasting their eggs over the substratum without digging a redd. The round whitefish showed no territorial or digging behavior, and individuals which had spawned did not have scars which would have been produced by digging. It was possible to recover eggs near the surface of the substratum. These observations are consistent with those for other stream-spawning whitefish species which do not dig redds (Lindroth 1957; Fabricius and Lindroth 1954).

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Otolith Age Validation of Greenland Halibut (*Reinhardtius hippoglossoides*)

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LEAR, W. H. AND T. K. PITT. 1975. Otolith age validation of Greenland halibut (*Reinhardtius hippoglossoides*). J. Fish. Res. Board Can. 32: 289–292.

Length and age distribution, together with evidence indicating the annual formation of an opaque and translucent hyaline zone on the otoliths of Greenland halibut (*Reinhardtius hippoglossoides*), point to the validity of using otoliths in age determination. The high incidence of opaque zones during July–October suggests that these fish grew fastest during this period. Young fish appeared to have a longer annual growth period than the older specimens.

LEAR, W. H., AND T. K. PITT. 1975. Otolith age validation of Greenland halibut (*Reinhardtius hippoglossoides*). J. Fish. Res. Board Can. 32: 289–292.

La distribution des longueurs et des âges, ainsi que la preuve de la formation annuelle d'une zone opaque et d'une zone translucide sur les otolithes du flétan du Groenland (*Reinhardtius hippoglossoides*) semblent appuyer la validité des otolithes pour la détermination de l'âge. La haute fréquence de zones opaques en juillet–octobre donne à croire que ces poissons croissent le plus rapidement durant cette période. Les jeunes semblent avoir une période de croissance annuelle plus longue que les poissons plus âgés.

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THE importance of a critical approach to ensure conformity of methods and results in age determination has been pointed out by Graham (1956), Pitt (1967), Smidt (1969), and Powles (1966).

Graham (1956) reviewed seven techniques for demonstrating the reliability of otoliths and scales for ageing fish. Two of these have been applied to validate the determination of age of Greenland halibut (*Reinhardtius hippoglossoides*) from otoliths, namely the Petersen method and the seasonal changes in the edge of the otolith.

The Petersen method which makes use of the polymodal nature of the length-frequency distributions of samples containing fish of more than one age-group is a useful validation technique but cannot be used to age individual fish. The seasonal change in the otolith does not validate a particular age or age-group but provides evidence of the seasonal changes in the otolith edge which produces the annulus or growth ring. The aim of this study is to demonstrate the validity of age determination of Greenland halibut from otoliths so as to provide a sound scientific basis for age interpretation and growth patterns which form the bases of stock assessments and the eventual establishment of quotas for the species.

The otolith method is preferred over the scale method because of the greater difficulty in determining age from scales even from the younger fish.

Methods and materials — The length and ageing material were collected from research cruises off eastern Newfoundland and Labrador during 1966–69. The sampling gear used was a standard No. 41.5 otter trawl with a 1⅝-inch nylon liner. When Greenland halibut were caught, they were measured and random samples were obtained for length, weight, sex and maturity, and otoliths.

The left sacculus otolith is more suitable for age reading because the apparent annuli are spaced more evenly and are more distinct. The otolith was ground on its convex surface by a fine-grit, revolving, horizontal carborundum stone powered by an electric sewing-machine motor. The otolith was then placed in a black watch glass containing ethyl alcohol, which made the hyaline zones more distinct. The ages were assigned by observing the prepared otoliths at 10× to 15× magnification. Interpretation of age was sometimes difficult, especially for fish over 10 yr old, when

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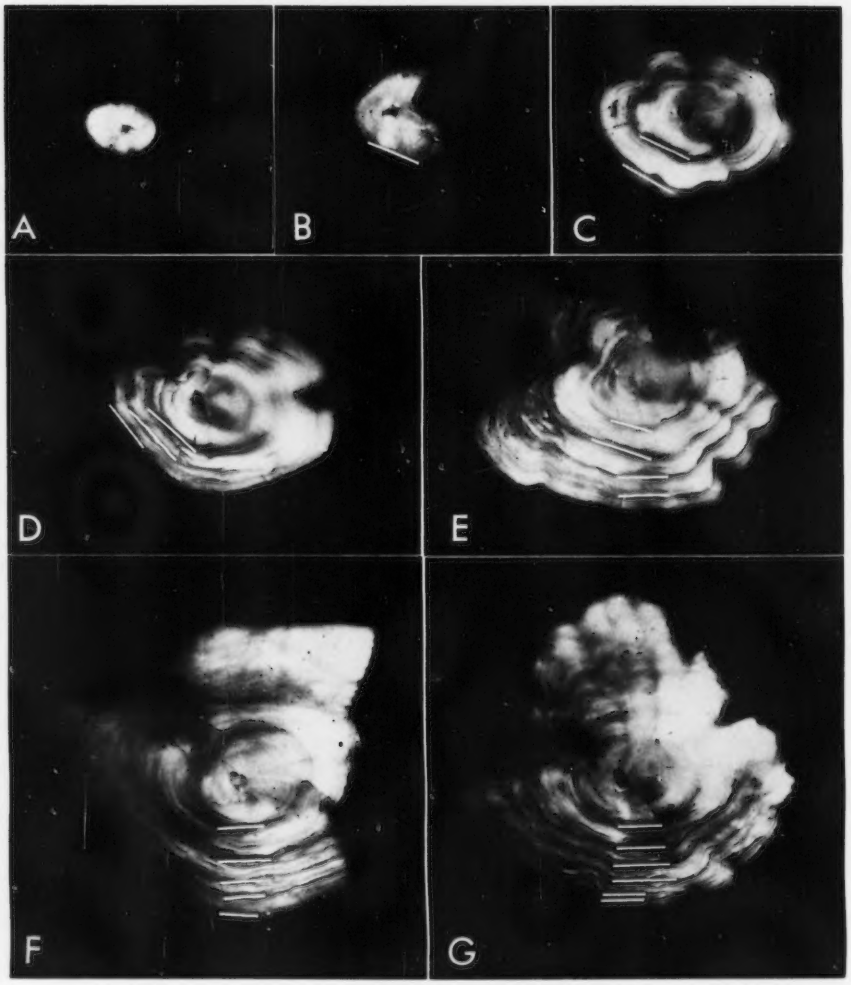

FIG. 1. Representative otoliths of the ages 0+–6 yr inclusive (×8). A, age 0+, 7.1 cm October 21, 1967; B, age 1, 12 cm April 23, 1967; C, age 2, 16 cm October 23, 1967; D, age 3, 21 cm October 23, 1967; E, age 4+, 33 cm October 30, 1967; F, age 5, 36 cm January 16, 1968; G, age 6, 41 cm January 16, 1968. The marks indicate the center of the hyaline zone of each annulus. All the otoliths except B are from the left side of the fish.

both hyaline and opaque zones are narrow and thus the ages of these older fish may be in error ± 1 or 2 yr.

Seasonal changes in otolith edge deposition were also recorded to give an indication of the seasonal growth pattern of the fish. Modal lengths of the otolith ages were compared with modes in length distribution, to validate the otolith ages from the Labrador Shelf. Also age distributions for 2 successive yr (1966–67) on Hamilton Inlet Bank were plotted graphically to illustrate the 1965 year-class dominance in each year. In addition 0+ age-group fish were collected from cod stomachs from Hamilton Inlet Bank and used as further support for otolith age validation. Representative otoliths of age-groups 0+–6 yr inclusive were photographed to show annual growth zones (Fig. 1).

Results — Length distributions of Greenland halibut sampled from Hamilton Inlet Bank during

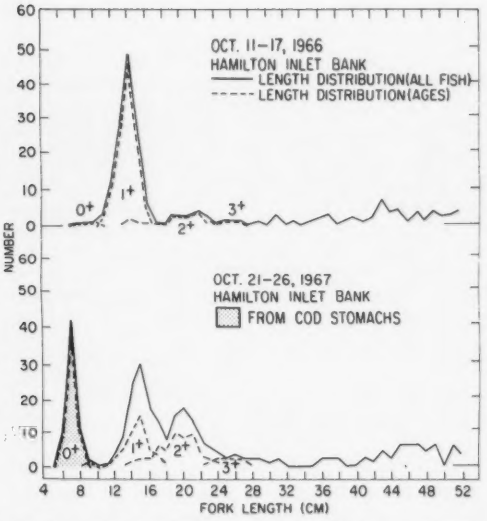

FIG. 2. Length distributions of otolith age-groups superimposed upon the length distributions of Greenland halibut (*Reinhardtius hippoglossoides*) from Hamilton Inlet Bank during October 11–17, 1966 and October 21–26, 1967.

October 11–17, 1966 and October 21–26, 1967 are distinctly polymodal for younger ages but not so in larger, older fish (Fig. 2). The smallest size at which a peak appears for the 1966 length distribution is 12–16 cm. Otoliths of these fish had a typically opaque central area with a narrow translucent band and an outer edge of opaque material which was interpreted as representing 1 full yr of growth plus an additional part of another year. This is supported by the 1967 length distribution which includes a sample of small Greenland halibut taken from cod stomachs. In some otoliths of the 0+ age-group, a very wavy translucent ring which was interpreted as a settling ring was observed. The polymodal nature of the length distribution becomes indistinct beyond age 2 probably because size of the faster-growing fish of one age-group overlaps size of slower-growing fish of the next age-group. The strong 1+ age-group in 1966 is also prominent as 2+ fish in 1967, whereas the 2+ age-group of 1966 is barely distinguishable as 3+ in 1967.

The otoliths of a few fish show opaque edges as early as April but the greatest proportion of fish with opaque otoliths occurs in July–October (Fig. 3). By November otoliths of some fish were beginning to show a narrow hyaline zone at the edge. Otoliths of younger fish (ages 1–6 yr inclusive) show an opaque zone at the otolith edge

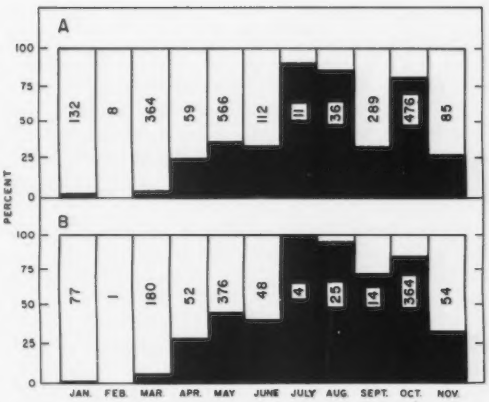

FIG. 3. Monthly incidence of opaque edge deposits on otoliths from all areas for: A, fish of all ages, and B, fish of age 0+–6 yr inclusive. Numbers of fish are shown.

earlier than those of older fish. In November the otoliths of younger fish indicated a greater proportion with hyaline zones than those of older fish. From 50 to 100% of the otoliths of fish of ages 1–4 show an opaque zone in the otolith edge in May and the percentage decreases with increasing age (Fig. 4). This suggests that the annual period of growth in old individuals is relatively shorter than for the younger fish (ages 1–6 inclusive).

Discussion — Jensen (1935) established that young larvae of Greenland halibut in Davis Strait were 10–18 mm long during April 7–May 9. Pelagic larvae were 17–28 mm around June 20, 19–30 mm on July 1, and 24–41 mm about July 7. The largest Greenland halibut larvae recorded by Jensen (1935) measured 34–57 mm during August 27–29. He also reported Greenland halibut larvae at 60–70 mm in cod stomachs at the beginning of September. These larvae were fish which had hatched the preceding winter and

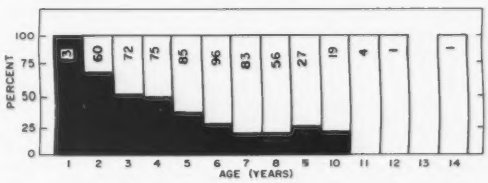

FIG. 4. Incidence of opaque edge deposits on otoliths during May. Numbers of fish are shown.

spring and were in the 0+ age-group. These results agree with the measurements of 65 larvae from cod stomachs from Hamilton Inlet Bank. The larvae in this case, on October 21, 1967, ranged in length from 5.9 to 7.7 cm with an average length of 6.9 cm. The length modes for the 1+ and 2+ age-groups compare with those of Smidt (1969) for Greenland halibut in West Greenland.

From the seasonal incidence of opaque and hyaline zones of otolith edges it is reasonable to conclude that one hyaline and one opaque zone are deposited each year. However, checks may occur between the annual hyaline zones and these may cause difficulty in the interpretation of age. These checks, however, can usually be recognized as accessories because they do not form a complete ring around the central area or because they are much narrower than the annual hyaline zone.

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Trace Analysis of Phenols in Water by Gas Chromatography

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MURRAY, D. A. J. 1975. Trace analysis of phenols in water by gas chromatography. J. Fish. Res. Board Can. 32: 292–294.

A method for analysis of low concentrations of phenols, cresols, and xylenols in water samples was developed. O-xylene was added to the sample as an internal standard and the sample was extracted once with chloroform to remove a portion of the total organic material present. The trimethylsilyl derivatives of the phenols were formed and analysis completed by gas chromatography. The method was rapid and required a minimum of sample manipulation. The lower limit of detection was 0.100 mg/liter for phenol, 0.025 mg/liter for cresols, and 0.050 mg/liter for xylenols.

MURRAY, D. A. J. 1975. Trace analysis of phenols in water by gas chromatography. J. Fish. Res. Board Can. 32: 292–294.

L'auteur a mis au point une méthode d'analyse de faibles concentrations de phénol, crésols et xylénols dans des échantillons d'eau. Il ajoute de l'O-xylène à l'échantillon comme étalon interne et soumet l'échantillon une fois à l'extraction au chloroforme pour enlever une portion de la matière organique totale présente. Les dérivés triméthylsilyliques des phénols sont formés, et l'analyse est complétée par chromatographie en phase gazeuse. La méthode est rapide et ne requiert qu'un minimum de manipulation de l'échantillon. La limite inférieure de détection est de 0.100 mg/litre pour le phénol, 0.025 mg/litre pour les crésols et 0.050 mg/litre pour les xylénols.

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IN the course of development work to provide an analytical method for tri-aryl phosphate esters and traces of their breakdown products in environment samples, the following technique was developed. The products of hydrolysis of Imol, a commercial lubricant, are phosphate and a mixture of phenolic substances. Analysis for phos-

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phate is simple, but since it is present in natural waters from many sources, work was concentrated on analysis of the phenolic fraction.

Standard colorimetric methods of analysis for phenols permit only the determination of total monohydric and polyhydric phenols. A gas-chromatographic method would separate the individual components and allow a more complete analysis. Baker and Malo (1967) determined phenols using gas chromatography by aqueous injection, but difficulties were encountered due to tailing and ghost peaks. Eichelberger et al. (1970) used an activated carbon column to collect and concentrate the phenols, followed by elution with chloroform. Mieure and Dietrich (1973) used methylene chloride to extract trace organic components from water and a gas chromatography–Mass Spectrometer combination for positive identification. Cooper and Wheatstone (1973) used multiple extractions with methyl iso-butyl ketone, followed by silylation and gas-chromatographic analysis using an internal standard for quantitation of phenols. Quinn and Meyers (1971) used three extractions with chloroform to recover organic acids from sea water, followed by gas-chromatographic analysis using an internal standard added to the sample before extraction.

The object of this work was to provide a rapid and sensitive method for the analysis of phenols in water samples.

Materials and methods — In the present method, an internal standard is added to the water samples followed by one solvent extraction. Since a ratio is established when the internal standard is added, and since a constant proportion of each organic compound is removed by a solvent extraction, only a portion of the total organic material present need be removed for analysis. The extract is concentrated, a derivative formed and analyzed by gas chromatography.

The chemicals used, phenol, cresols, and xylenols were of the highest purity available from Eastman Organic Chemicals. Tri-Sil concentrate was obtained from Pierce Chemicals and the chloroform was from Fisher Scientific. Imol was obtained from Imperial Oil Co. Ltd.

Gas chromatography

A dual column Perkin Elmer 900 Gas Chromatograph with Flame ionisation detectors was used in a single column mode, in conjunction with an Infotronics C.R.S. 208 Digital Integrator and Hewlett Packard 1 mv, recorder. The columns used were 8 ft × ⅛ inch OD stainless steel and were packed with 5% Imol coated on Chromosorb W., A.W., and D.M.C.S. treated. The columns were used isothermally at 120 C, or programmed from 80 to 120 C at 4°/min to provide a better separation.

Operating conditions

Injector temp	150 C
Manifold and detector temp	150 C
Carrier gas (nitrogen)	25 ml/min.
Hydrogen	35 ml/min.
Air	400 ml/min.

A 1-liter sample of water containing known amounts of phenol, cresols, or xylenols was placed in a 1 liter separatory funnel and 5 μl of ortho-xylene carefully added by syringe as an internal standard. The sample was shaken and a single solvent extraction made by shaking with 50 ml of chloroform for 2 min. When the solvent layer had separated it was transferred to a 100-ml pear-shaped flask with a ground glass joint and reduced to about 1 ml on a rotary evaporator at 25 C under vacuum. Care must be taken to avoid losses of volatile material at this stage by preventing the flask from drying out. The sample was treated with 250 μl of Tri-Sil concentrate at room temperature for 30 min to form the trimethylsilyl derivatives. It was necessary to leave the xylenols overnight to react possibly because of steric hindrance as found by Klebe et al. (1966). The solution of the derivatives was injected into the Gas Chromatograph, the peak areas measured, and the ratios of the phenols to the internal standard calculated. The ratios were plotted against the original amount of phenol, cresol, or xylenol added and a linear relationship was found in all cases over the range 0–10 mg/liter. The calibration factors were calculated as in Table 1.

Results and discussion — The gas-chromatographic analysis follows the technique of Cooper and Wheatstone in that the trimethylsilyl derivatives are formed using Tri-Sil concentrate as recommended by Pierce Chemical Co. (1972). The

TABLE 1. Calibration factors in R = KC where R is ratio to a constant amount of internal standard and C is concentration in milligrams/liter.

	K
phenol	.027
m-cresol	.100
p-cresol	.104
3:5 dimethylphenol	.135
o-cresol	.136
3:4 dimethylphenol	.143
2:3 2:5 dimethylphenol	.178
2:4 2:6 dimethylphenol	.192
2 ethylphenol	.230

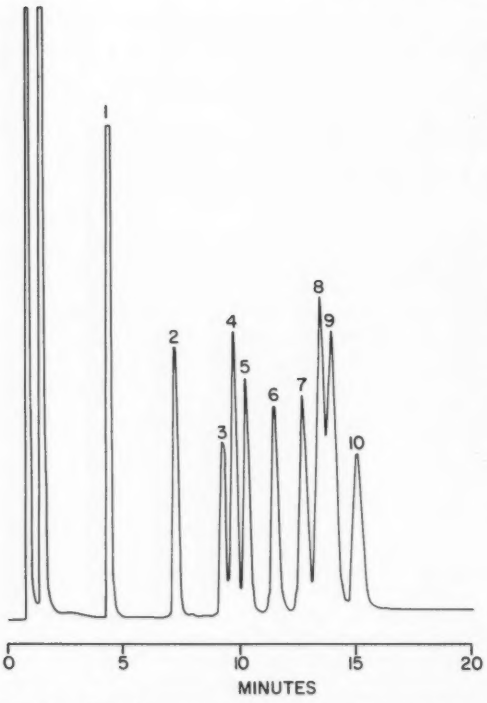

FIG. 1. Gas-chromatographic temperature program, 4°/min, 80–120 C. 1, o-xylene; 2, phenol; 3, o-cresol; 4, m-cresol; 5, p-cresol; 6, 2:5 xylenol; 7, 2:4 xylenol, 3:5 xylenol; 8, 2:6 xylenol; 9, 2:3 xylenol; 10, 3:4 xylenol. Peak 7 contains both compounds listed.

liquid phase, tri-xylenyl phosphate, of Cooper and Wheatstone was not available, so Imol, mainly tri-tolyl phosphate was used. Phenol, the cresols, and the xylenols were separated on a 5% Imol column (Fig. 1).

Percent extraction from the 1-liter sample was: phenol, 20; m-cresol, 39; p-cresol, 40; o-cresol, 48; 3:4 xylenol, 81; 2:6 xylenol, 83; o-xylene, 95. Phenol is the most hydrophilic and its extraction was the lowest. To achieve a 90% recovery of phenol, more than 10 extractions would be required. Once a ratio to the internal standard has been established, only a portion of the total organic material need be extracted and only one solvent extraction is necessary. When the chloroform is evaporated to a small volume, the net effect is a 200 times concentration for phenol.

Extracts were made of a hard water, a soft water, a local river water, and a final sewage effluent sample from an activated sludge treatment plant. No cleanup was used. No interfering peaks were found on the chromatograms. When these natural samples were spiked with 5- and 10-mg/liter amounts of cresols and xylenols the recoveries were 98–100%.

This technique will quantitatively and specifically measure as little as 0.100 mg/liter of phenol, 0.025 mg/liter of cresols, and 0.050 mg/liter of xylenols in a water sample, with a relative standard deviation of ± 4.48% at a concentration of 5 mg/liter. Regression analysis showed a correlation coefficient between the ratio to the internal standard and the concentration of 0.997 for phenol, 0.999 for the cresols and 0.998 for the xylenols. Since only one solvent extraction was sufficient, there was a significant saving in time and labor per sample. The solvent does not have to be ultra-high purity and the method was both rapid and convenient to use.

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Cytotaxonomy of the Salmonid Fish *Stenodus leucichthys*

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BOOKE, H. E. 1975. Cytotaxonomy of the salmonid fish *Stenodus leucichthys*. J. Fish. Res. Board Can. 32: 295–296.

The karyotype for the coregonine fish *Stenodus leucichthys* (determined from squashed blastula preparations) has a diploid number of 74, consisting of 20 metacentric, 14 submetacentric, and 40 acrocentric chromosomes and a total arm number of 108. This karyotype is very similar to that of *Coregonus clupeaformis*. *Stenodus* was probably derived from *Coregonus* by means of Robertsonian translocations during the early stage of coregonine evolution.

BOOKE, H. E. 1975. Cytotaxonomy of the salmonid fish *Stenodus leucichthys*. J. Fish. Res. Board Can. 32: 295–296.

Le caryotype du Coregoninae, *Stenodus leucichthys*, (déterminé sur des préparations de blastule écrasée) est formé d'un nombre diploïde de 74 chromosomes: 20 métacentriques, 14 submétacentriques et 40 acrocentriques, avec un nombre de bras total de 108. Ce caryotype ressemble beaucoup à celui de *Coregonus clupeaformis*. *Stenodus* est probablement dérivé de *Coregonus* par translocations robertsoniennes au début de l'évolution des Coregoninae.

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THE coregonine *Stenodus leucichthys* (Güldenstädt), the inconnu, the largest whitefish and the only one that is primarily piscivorous, has been discussed as a form of whitefish (sensu lato) that was either derived very early in salmonid phylogeny or derived more recently (see Norden 1961). Cavender and Miller (1972) have indicated that this species has many primitive features shared by both the Coregoninae and the Salmoninae, implying a closer relationship between *Stenodus* and the Salmoninae than was considered by Norden. To help resolve the systematics of the monotypic genus *Stenodus* within the subfamily Coregoninae, family Salmonidae, I have determined its karyotype to add to and reassess cytogenetic data previously gathered (Booke 1968) on this and other North American coregonine species.

Methods — Inconnu were caught in spawning condition in the Kuskokwim River and the Chatanika River (Upper Yukon River), Alaska on October 1, 1971, and October 1, 1972, respectively. Two Kuskokwim adult study specimens are deposited in the Museum of Zoology of The University of Michigan (UMMZ 191861, 647 and 678 mm SL).

The karyotype was determined from blastulae (squash preparation) as described by Booke (1968). The 20 counts made to obtain the diploid number did not vary, nor was sexual dimorphism noted in the karyotype. The fertilized eggs were incubated to the 42 Temperature Unit (TU) stage (one TU was gained for every 0.56 degrees above 0 C per 24 h of incubation time). The 34–38 TU stages gave the best preparations for chromosome spread and size.

Chromosome numbers and discussion — The diploid number for *S. leucichthys* is 74, with 20 metacentric, 14 submetacentric, and 40 acrocentric chromosomes and a total arm number of 108 (Fig. 1). It is possible, through the means of translocations, that the karyotype of *Stenodus* was derived from that of *Coregonus clupeaformis* or its progenitor. I reported (1968) the karyotype for *C. clupeaformis* as having a diploid number of 80, consisting of 20 metacentric, 8 submetacentric, and 52 acrocentric chromosomes and an arm number of 108. The karyotype of *S. leucichthys* closely resembles that of *C. clupeaformis* in morphology. Robertsonian fusions among 12 acrocentrics to form 6 submetacentrics are all that need occur to produce the karyotype of *Stenodus*.

In a previous study of the coregonine fishes of the St. Lawrence Great Lakes (Booke 1968), I reported that the guanine plus cytosine percent and the DNA-Feulgen amount of DNA for most species in the genus *Coregonus* were the same as for *S. leucichthys*. These data were interpreted as support for Norden's (1961) osteological evidence indicating that *Stenodus* was closely allied

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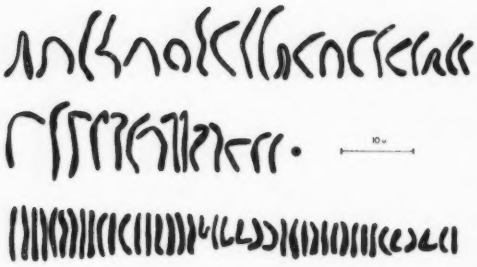

FIG. 1. The karyotype of *Stenodus leucichthys*. The dots separate metacentric, submetacentric, and acrocentric chromosomes, in that order.

to *Coregonus*. In addition to these molecular data and Norden's (1961) morphological data available for *S. leucichthys*, this species' karyotype can also be used to assess its relationship to the other coregonine genus, *Prosopium*.

I make the inference, based on specific chromosome changes that have occurred, that *Prosopium* and *Stenodus* are old in terms of coregonine evolution. For those salmonid species that make up morphologically distinct genera, chromosome numbers have changed and become species specific in time (Booke 1968, 1974; Roberts 1967). This change has not occurred in species of *Coregonus*, which have maintained (for the most part) the constant chromosome number and highly variable phenotypes that would indicate early species incipience. The other coregonine genera, *Prosopium* and *Stenodus*, have species-specific chromosome numbers.

I believe that the lack of parr marks and possession of a double nasal flap (both characters absent in *Coregonus* but present in *Prosopium*), and a karyotype which could be derived from a form of *Coregonus*, are strong evidence that *Stenodus* is a distinct genus closely related to *Coregonus* and was derived from it in an early stage of coregonine evolution after *Prosopium* was established. Behnke (1972) also has indicated that speciation of *Stenodus* was subsequent to the *Prosopium* stage (the earliest stage) of coregonine evolution. In contrast, Cavender and Miller (1972) implied that *Stenodus* might have been derived much earlier in coregonine phylogeny. Their reasoning was based on a comparison of *Stenodus* with their newly described fossil salmonid, *Smilodonichthys rastrosus*, which lacked maxillary teeth, a character that was previously thought to be basically coregonine and not evident in the salmonine line. They also showed that *Stenodus* shares a number of primitive features found in both the Salmoninae and the Coregoninae. However, these features could be interpreted as examples of parallel evolution between these lines. The lack of maxillary teeth in *Smilodonichthys rastrosus* may be a nonconstant character that occurred only in this line in the Salmoninae.

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Inhibition of Formation of Free Fatty Acids in Cod Liver with Sodium Hypochlorite Solution (Javex)

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KE, P. J., AND R. G. ACKMAN. 1975. Inhibition of formation of free fatty acids in cod liver with sodium hypochlorite solution (Javex). J. Fish. Res. Board Can. 32: 297–300.

Inexpensive active chlorine from commercially available Javex has been investigated for the preservation of cod livers at room temperature. Both homogenized and chopped liver samples were treated with preservative solution containing active chlorine concentrations of from 0.01 to 1.35%. After 7 days the formation of free fatty acids in cod livers by hydrolysis of lipids was inhibited by as much as 82 and 72%, respectively, for homogenized and chopped livers. Autolysis was effectively reduced by increasing the concentration of active chlorine, but accelerated markedly after a 10-day storage period. The peroxide value increased slowly in the liver treated with active chlorine solution (0.79%) from 0 to 3.2 meq/kg in a 7-day period and then reached 6.0 after 12 days of storage. About one-half of vitamin A content in the livers was destroyed during a 7-day treatment, but only 24% of vitamin D was lost. Since synthetic vitamin A can be cheaply added to the oil, these results show that active chlorine can be used as a cheap preservative in the fish liver industry, and provide a basis for the development of multiple component preservatives for livers.

KE, P. J., AND R. G. ACKMAN. 1975. Inhibition of formation of free fatty acids in cod liver with sodium hypochlorite solution (Javex). J. Fish. Res. Board Can. 32: 297–300.

Les auteurs ont étudié le chlore actif peu dispendieux du Javex de commerce comme agent préservatif des foies de morue à la température du laboratoire. Des échantillons de foies, homogénéisés aussi bien que hachés, ont été traités avec une solution contenant des concentrations de chlore actif allant de 0.01 à 1.35%. Au bout de 7 jours, la formation d'acides gras libres dans les foies de morue par hydrolyse des lipides est paralysée dans des proportions allant jusqu'à 82% pour les foies homogénéisés et 72% pour les foies hachés. Une augmentation de concentration de chlore actif réduit effectivement l'autolyse, mais cette dernière augmente notablement après entreposage de 10 jours. Dans les foies traités avec une solution de chlore actif (0.79%), la valeur du peroxyde augmente lentement de 0 à 3.2 méq/kg sur une période de 7 jours, pour atteindre 6.0 après 12 jours d'entreposage. Environ la moitié du contenu en vitamine A des foies est détruite au cours du traitement de 7 jours, mais la vitamine D ne fait que se décomposer à près de 24%. Comme la vitamine A synthétique peut, à peu de frais, supplanter l'huile, ces résultats démontrent que le chlore actif peut être utilisé comme préservatif peu dispendieux dans l'industrie des foies de poisson et servir de base au développement de préservatifs à plusieurs composants pour les matières tirées du foie.

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THE free fatty acids (FFA) in fish liver oils generally present a serious quality problem themselves, if in high proportion, and are an index of degradation affecting other components such as vitamins (Volkova et al. 1971 and Dolbish 1967). Preservation of cod livers is often desirable while enough cod livers are being accumulated for rendering (Butler 1948). The problems in preservation and treatment of cod livers have been briefly reviewed by Guttmann and Vandenheuvel (1958) and Jangaard and Power (1966). The sterilization of cod livers by gamma-ray irradiation has recently been investigated, but this cannot prevent hydrolytic decomposition of triglycerides by enzymatic processes (Ackman et al. 1966). Isopropanol has been studied as a preservative of raw materials for fish protein concentrate, and can inhibit free fatty acid formation to a certain degree in cod livers (Jangaard and Power 1966; Guttmann et al. 1967). Jangaard and Power (1966) also pointed out that chlortetracycline (Acronize) would be of no value against enzyme activity if used alone, even in the very high concentration of 15 ppm.

Formation of free fatty acids in vegetable oils

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TABLE 1. Percentage of FFA formation and inhibition in cod livers stored for 7 days at various active chlorine solution.

Active Cl, % in solution added	Cut livers		Homogenized livers	
	FFA %	Inhibition %	FFA %	Inhibition %
0.00 (control)	17.5	–	10.7	–
0.01	14.0	20.0	9.7	9.1
0.05	13.2	25.0	9.1	15.0
0.26	8.9	49.0	7.4	31.0
0.53	6.6	62.0	3.9	63.0
0.79	6.6	63.0	3.6	66.0
1.35	4.8	72.0	1.9	82.0

and animal fats was inhibited by treating the pure fats with a solution of active chlorine (Norris and Grettie 1967; Tribble and Rondenet 1967). By comparison with most oils and fats, cod liver is very complicated fatty raw material to be preserved for its oil without hydrolytic decomposition to form free fatty acids (Guttmann and Vandenheuvel 1958; Jangaard and Power 1966; Ackman et al. 1966). The main concern of the present study is a demonstration of the effect of sodium hypochlorite solution, as a form of readily available and inexpensive active chlorine, on hydrolysis of fats in cod livers where either or both of autolytic or bacterial hydrolytic activity is present.

Methods — Household Javex (trademark of Bristol-Myers Ltd., and containing 5.3% active chlorine as sodium hypochlorite) was diluted with distilled water to give various concentrations of stock solution. Atlantic cod (*Gadus morhua*) taken at Terrence Bay, N.S., and transported alive to the laboratory in Halifax provided a source of fresh livers. Several livers were removed from fish killed under clean but not sterile conditions, and the livers were either cut in small pieces (about pea size) which were mixed by hand, or mixed after maceration in a household meat grinder.

Seven 15-g portions of these livers were placed in individual cork-stoppered standard oil sample bottles (approximately 100 ml capacity). Water (10 ml) or different stock solutions of household Javex (10 ml) were added to respective bottles. Storage to permit the autolytic and hydrolytic processes was at room temperature (23–30 C). After 7 days, oils and fatty acids were recovered by extraction with hexane and analyzed for free fatty acids by titrimetric methods in Ca-5a-40 of the American Oil Chemists' Society (AOCS 1972). Blank titrations for various stock solutions containing hypochlorite showed a need for minor compensating corrections and these have been applied to the results.

In a further study, a set of four bottles of homogenized liver samples were treated similarly with 0.79% active chlorine solution. An additional set of liver samples treated with distilled water was used as the control to compare the effect of active chlorine on lipid oxidation in terms of peroxide value (POV) which was determined by AOCS method (Cd-8-53, AOCS, 1972). Oils from cod livers were extracted as in the previous experiment at intervals for study. For oils from cod livers treated with 0.79% chlorine, GLC analyses of fatty acids were made by previously developed procedure (Ackman and Eaton 1971), and vitamin A and D were estimated spectrophotometrically by the procedures of Thies and Steinigen (1966), and Doboszynska (1971), respectively.

Results and discussion — The observed inhibitions of FFA production caused by active chlorine in the various stock solutions ranging in concentration from 0.01 to 1.35% were, respectively, 20.0–72.0% for cut livers and 0.00–82.0% for homogenized liver samples (Table 1). Although the percent inhibition of FFA production is higher for chopped livers than for homogenized samples at the chlorine concentration below 0.5%, the fat content of the livers used is a variable affecting FFA formation. Norris and Grettie (1967) have discussed the inhibition of FFA formation with an oxidizing agent like hydrogen peroxide. This is completely ineffective since it is rapidly destroyed by the enzyme catalase present in the tissue. It is quite possible that a more readily accessible non-fatty constituent in homogenized livers would bind the limited amount of chlorine at the lower concentrations and reduce the amount available to stop enzyme action. Chopping appears to be quite adequate in permitting access of active chlorine to the liver fat.

The extent of inhibition of FFA formation was proportional to concentration for two different chlorine stock solutions in the first 2 wk as shown in Fig. 1. The FFA formation was effectively reduced from 13 to 5 or 2% by increasing the concentration of active chlorine from 0.53 to 1.35% in the first 10 days of storage (see Curves A, B and C in Fig. 1), but beyond that time FFA formed rapidly, especially at lower concentration of chlorine. The results are comparable to those in a previous study (Norris and Grettie 1967).

TABLE 2. Variations of fatty acid composition and vitamin contents in homogenized cod livers with active chlorine solution (0.79%).

Tests	Stored days 0	3	7	12
FFA %	–	1.2	3.2	6.0
POV (meq/kg oil)	0	0.2	2.72 (1.5[a])	4.8 (3.9[a])
Vitamin A (IU/g oil)	5090	–	2650	–
Vitamin D (IU/g oil)	340	–	259	–
Fatty acids (mole %)				
Saturates	16.7	–	17.5	18.1
C14:0 + C16:0 + C18:0[b]	16.1	–	16.9	17.6
Monoenes	60.2	–	59.6	59.4
Polyenes	23.1	–	22.9	22.4
C18:4 + C20:5 + C22:6[b]	17.2	–	15.9	15.5
Polyene index				
C18:4 + C20:5 + C22:6 / C14:0 + C16:0 + C18:0	1.07	–	0.94	0.88

[a]Data for the control samples treated without adding active chlorine solution.
[b]Shorthand notation gives chain length: number of double bonds.

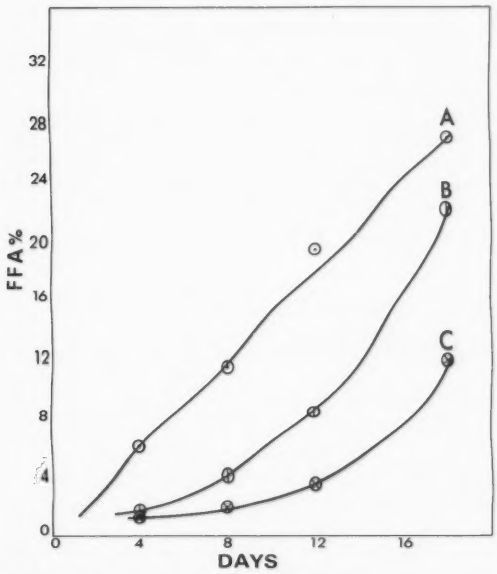

FIG. 1. Effect of active chlorine treatment on the FFA formation in cod liver at various times (curves *A, B, and C refer to the percentage of active chloride* added in solutions of 0, 0.53, and 1.35%, respectively).

Lipid recovery from all samples treated with different chlorine concentrations remained unaffected. Peroxides from lipid oxidation formed slowly during the period of 7 days and then increased rapidly when it went beyond its induction period and that has been reported previously for other lipid oxidation systems (Schultz et al. 1962). Peroxide value in homogenized cod liver treated with 0.79% active chlorine solution for a storage period from 0 to 12 days was increased from 0 to 4.8 (Table 2). For the controls (with no active chlorine used) lipids in cod liver were also slowly oxidized with the POV of 1.5 and 3.9 meq/kg after 7 and 12 days of storage (Table 2, data in brackets). In comparison from two sets of results, oxidative rancidity in cod liver treated with active chlorine was slightly increased, even though this is still within acceptable limits in terms of oil quality. Polyunsaturated, monounsaturated, and saturated fatty acids from the oil of cod liver treated with active chlorine are presented in Table 2 for comparison (Swern 1964). The polyene index was also computed and shows the same tendency as POV data (Table 2). Since polyunsaturated fatty acids are particularly susceptible to the action of air, the slight decrease of polyenes may be mainly due to the oxidation of lipids. Vitamin A content of cod livers was reduced by nearly 50% after 7 days of treatment with 0.79% active chlorine solution, whereas only 24% of vitamin D was lost (Table 2). However, synthetic vitamin A preparations are available cheaply and in quantity, and they have been used to supplement the oil products (Brody 1965).

We have shown that active chlorine is definitely effective in inhibiting the FFA production more than 72% in 7 days of storage at room temperature, which is beyond inhibition reasonably attributable to the destruction of bacteria alone since neither Acronize treatment nor irradiation sterilization demonstrated any effect on lipid

hydrolysis (Ackman et al. 1966; Merrit 1966). Lower temperatures could be beneficial but were not included in this study.

Unfortunately, no treatment with a single chemical preservative is known to work satisfactorily to limit the FFA formation as much as desirable. Basically the presence of both fat and aqueous phases hinders distribution of most effective agents. A multiple-component preservative based on or including sodium hypochlorite solutions should be developed to inhibit effectively both bacterial and enzymatic processes in cod livers.

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Experimental Study of Galvanonarcosis for Rainbow Trout (*Salmo gairdneri*) Immobilization

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KYNARD, B., AND E. LONSDALE. 1975. Experimental study of galvanonarcosis for rainbow trout (*Salmo gairdneri*) immobilization. J. Fish. Res. Board Can. 32: 300–302.

Galvanonarcosis treatments had no significant effect on growth or photonegative behavior of rainbow trout (*Salmo gairdneri*). The data indicate the potential usefulness of the technique for immobilizing fish.

KYNARD, B., AND E. LONSDALE. 1975. Experimental study of galvanonarcosis for rainbow trout (*Salmo gairdneri*) immobilization. J. Fish. Res. Board Can. 32: 300–302.

La galvanonarcose n'affecte pas de façon significative la croissance ou le comportement photonégatif de la truite arc-en-ciel (*Salmo gairdneri*). Les données démontrent l'utilité potentielle de la méthode pour immobiliser les poissons.

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GALVANONARCOSIS is defined as the reaction of fish to an uninterrupted direct electric current whereby the fish roll over onto one side, facing the anode and are incapable of movement (Halsband 1967). Although there is general disagreement on the mechanism by which the paralysis occurs (Scheminzhy and Kollensperger 1938; Hartley 1967; Vibert 1963; and C. Volz personal communication), it is generally agreed that galvanonarcosis is the safest and least exhausting manner of immobilizing fish with electricity. For this reason, the present study evaluates 1) the usefulness of a steady DC current to immobilize rainbow trout (*Salmo gairdneri*) and 2) the effects of the treatment on subsequent fish growth and perception of their environment.

Materials and methods — Yearling rainbow trout approximately 12 cm long were obtained from the Arizona Game and Fish Hatchery at Page Springs, Ariz. Prior to testing, fish were held in a large outdoor pool for 30 days and were fed daily with Purina Fish Chow.

Aerated tap water (temperature 13–21 C; specific conductivity 450 $\mu\Omega$/cm) that had been aged for 1 wk was used in all experiments.

During treatments, fish were held in a plexiglass aquarium (36 cm × 20 cm × 22 cm). Electrodes of sheet aluminum (20 cm × 22 cm) at each end of the aquarium created a nearly uniform electric field throughout the length of the aquarium. Polarity of the current was reversed every 30 min during experiments to prevent alteration of water quality through the electrolysis process.

Narcosis was produced when fish were subjected to a minimum of 10 V DC from an EICO Model #1020 power supply. Using DC to produce the field resulted in a 1-V drop at the interface between the negative plate and the water. This resulted in a field intensity of .25 V/cm. To determine the approximate head to tail voltage, which is the primary determinate in galvanonarcosis, we use

$$V_{H\text{-}T} = \bar{E}(L)$$

volts where E is the electric field intensity and L is the length of the fish in centimeters. The experimental fish were approximately 12 cm long which gives a head to tail voltage of 3 V. This agrees closely with the values determined by other investigators (Edwards and Higgins 1973).

To determine the effect of electrical treatments on growth, four groups of 30 fish each (15 experimental — 15 control) were tested at 1, 2, 4, and 6 h of galvanonarcosis on February 26 and 27, 1974. Control fish were held in an equivalent aquarium but were not subjected to an electrical current. Within 48 h after treatment, fish were individually weighed ± 1 g on a split-beam balance. Each of the eight groups was held separately in 1600-liter containers and fed approximately 2% of body weight/day until the experiment was terminated after 25 days when the fish were again weighed.

The effect of narcosis treatments on rainbow trout growth for the 25-day period was evaluated by *t*-test. Since there was no difference in initial mean weights between experimental and control fish for each test time, a similar comparison of the final mean weights was performed to reveal any growth difference that resulted from the treatment.

The possible effects of narcosis treatment on fish behavior was evaluated by comparing the photonegative responses of treated and control fish (yearling rainbow trout are photonegative, McCrimmon and Kwain 1966). The test environment was a pool 2.1 m² with water 50 cm deep. One-half of the pool was shaded by a wooden cover and one-half was exposed to direct sunlight. Percent transmittance of surface light at the bottom of the pool was 7% in the shaded area and 91% in the illuminated area.

Twenty-four fish were tested after narcosis treatment for photonegative response and 24 served as controls. Fish were tested in groups of six with two groups narcotized for 1 h and two groups for 3 h. After treatment, fish were immediately released into the center of the pool and scored for 1) initial choice of illuminated or shaded areas, and 2) subsequent preference of habitat for 15 min. Preference was recorded as the number of fish in each area at 1-min intervals for 15 min. Chi-square was used to compare the observed and expected frequencies of control and experimental fish. In the 15-min preference tests, the 12 control fish and 12 experimental fish for each test time (1 h and 4 h) were compared using the total number of fish that were counted in each habitat for all 1-min intervals.

Results and discussion — Fish were easily immobilized using the previously described techniques. In actual practice, the voltage was quickly increased to 20 V to cause immediate paralysis and reduce fright swimming. After 1 min at 20 V, trout could be held immobile at 10 V except for occasional short bouts of disoriented forced swimming.

Galvanonarcosis treatments appeared to be safe, with only two fish in the 6-h tests lost to mortality. In general, fish treated for 1 and 2 h recovered instantaneously as indicated by their resumption of swimming and feeding. Fish narcotized for 4 and 6 h had increased difficulty resuming swimming activity. Normal swimming activity and feeding were resumed within 12 h even by 6-h narcotized fish.

The *t*-test comparisons of experimental and control fish indicate there was no effect of galvanonarcosis on fish growth (Table 1). This seems to be the case although an accidental fish kill on March 23 eliminated some of the fish from the evaluation. Similar results for rainbow trout treated with pulsating DC current were obtained by Maxfield et al. (1971).

TABLE 1. Effect of galvanonarcosis immobilization on rainbow trout (*Salmo gairdneri*) growth. Sample size is in parentheses. NS = non significant.

Treatment time (*h*)	Experimental $\bar{X}$ wt (g)	Control $\bar{X}$ wt (g)	*t*-value
	Feb. 26, 27		
1	33.0 (15)	33.8 (15)	.17 NS
2	33.8 (15)	31.1 (14)	.57 NS
4	30.2 (15)	34.3 (15)	1.07 NS
6	34.6 (14)	30.5 (15)	1.07 NS
	Mar. 23		
2	40.1 (15)	34.8 (7)	1.0 NS
4	36.9 (15)	40.3 (14)	.75 NS
6	38.7 (13)	40.7 (7)	.87 NS

Galvanonarcosis had no effect on the photonegative response of rainbow trout. The initial takeoff direction of all control and narcotized fish was the same — they were photonegative. Control and experimental fish also did not differ in their strong photonegative response during the 15-min habitat preference period (1-h experiment, $\chi^2_{(1)} = .17$; 3-h experiment $\chi^2_{(1)} = 1.6$).

While under the influence of narcosis, fish experienced a decrease in ventilation rate (number of opercular beats/min) as counted by eye. For a random sample of five fish immobilized for 2 and 4 h and their controls, the ventilation rates were:

	Control fish % decrease	Experimental fish % decrease
2 h	22.1	44.8
4 h	17.6	51.6

This decrease in ventilation rate might allow the immobilization of large numbers of fish under crowded conditions that have a low level of dissolved oxygen.

Our data indicate that galvanonarcosis has promise as an immobilization technique in fisheries. It has already been used successfully for tagging adult Atlantic salmon, *Salmo salar*, in England (Hartley 1967). Narcotized fish in our experiments were easily handled and could have been tagged, finclipped, measured, etc. Rainbow trout that were narcotized for up to 2 h recovered quickly and suffered no deleterious effects that could be detected in this study. Besides the favorable indications from our experiments, the dollar costs of immobilizing fish with electricity should be quite competitive with the widely used chemical anesthetics.

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Adjustment of Buoyancy by Tadpole Madtom, *Noturus gyrinus*, and Black Bullhead, *Ictalurus melas*, in Response to a Change in Water Velocity

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MACHNIAK, K., AND J. H. GEE. 1975. Adjustment of buoyancy by tadpole madtom, *Noturus gyrinus*, and black bullhead, *Ictalurus melas*, in response to a change in water velocity. J. Fish. Res. Board Can. 32: 303–307.

Tadopole madtom (*Noturus gyrinus*) and black bullhead (*Ictalurus melas*) adjusted buoyancy by altering swimbladder volume becoming less buoyant in current than in still. Extent of adjustment was dependent on size. Tadpole madtom adjusted buoyancy within 96–192 h but partial adjustment occurred within 12–48 h. Year 0 fish made a more rapid decrease in buoyancy than did year 1 or 2. As buoyancy was decreased year 0 and 1 tadpole madtom showed significant changes in internal pressure of swimbladder gases. Black bullhead secreted gases into the swimbladder to increase buoyancy while tadpole madtom showed no such ability.

MACHNIAK, K., AND J. H. GEE. 1975. Adjustment of buoyancy by tadpole madtom, *Noturus gyrinus*, and black bullhead, *Ictalurus melas*, in response to a change in water velocity. J. Fish. Res. Board Can. 32: 303–307.

Le chat-fou brun (*Noturus gyrinus*) et la barbotte noire (*Ictalurus melas*) règlent leur flottabilité en changeant le volume de leur vessie gazeuse, et leur flottabilité est moindre en eau courante qu'en eau morte. Le degré de compensation dépend de la taille. Le chat-four brun règle sa flottabilité en dedans de 96–192 h, mais il y a réglage partiel en dedans de 12–48 h. Les poissons d'âge 0 réduisent leur flottabilité plus rapidement que les poissons d'âge 1 ou 2. A mesure que la flottabilité diminue, les chats-fous bruns d'âge 0 et 1 montrent des changements significatifs de pression des gaz dans la vessie gazeuse. La barbotte noire secrète des gaz dans sa vessie gazeuse pour augmenter sa flottabilité, alors que le chat-fou brun ne démontre pas d'aptitude semblable.

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THE ability of fish to increase buoyancy when still water is encountered and to reduce it as water velocity increases is an important adaptation to streams and rivers where water currents are variable. Although such adjustment is common amongst temperate stream fishes (Saunders 1965; Neave et al. 1966; Pinder and Eales 1969; Gee 1968, 1970; Gee et al. 1974) little is known of its rate and associated changes in internal pressure of swimbladder gases. This study on tadpole madtom (*Noturus gyrinus*) and black bullhead (*Ictalurus melas*) seeks to determine the extent and rate of adjustment of buoyancy, variation in internal pressure of swimbladder gases during such adjustment, and ability to secrete gases into the swimbladder.

Materials and methods — Tadpole madtom and black bullhead were collected from the Rat and La Salle rivers (Manitoba), respectively, during May to August in 1971 and 1972. They were held in the laboratory under a normal photoperiod in water of 21 ± 2 C.

To determine extent of adjustment of buoyancy, batches of 4–10 fish were placed in aquaria of still water or current for 8–10 days. Large aquaria (122 × 61 × 61 cm) were used to hold fish exceeding 150 mm total length while smaller aquaria (92 × 46 × 46 cm) were used for smaller fish. Current 20–35 cm/s) was created using stream tanks of a design similar to that of Gee and Bartnik (1969).

Fish were immobilized in a solution of tricane methanesulfonate and swimbladder volume (SBV; ± 0.001 ml), weight of gas-free fish in water (W; ± 0.001 g), and volume of gases released from the swimbladder at atmospheric pressure (VGR; ± 0.001 ml for fish < 110 mm TL; ± 0.1 ml for fish > 110 mm TL) were determined using the procedure of Gee (1970). Flotation pressure (SBV/W), a measure of buoyancy, and internal pressure of swimbladder gases (VGR/SBV) could be determined.

To describe rate of decrease in buoyancy and variation in internal pressure of gases during adjustment in tadpole madtom, 72 fish were held for 8 days in still water. Flotation and internal pressure of gases were recorded for eight fish (0 h). Then the remaining fish were exposed to current and examined in groups of eight after either 1, 3, 6, 12, 24, 48, 96, or

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192 h. Rate of decrease in buoyancy and associated changes in internal pressure were determined by holding 72 tadpole madtom in current for 8 days and groups of eight fish were examined at the above times in still water. This procedure was carried out on year 0 (24–41 mm), year 1 (44–59 mm), and year 2 (61–101 mm) to determine if rate of adjustment of buoyancy varied with age (size) of fish.

To induce gas secretion, swimbladder volume was reduced in both species by exposing fishes to a reduction in pressure causing swimbladder gases to be expelled through the pneumatic duct. Secretion of gases would be indicated if buoyancy was increased when fishes were denied access to the surface. Fish held individually in small plastic vials to restrict movement were weighed in water, exposed to a vacuum of 13–30 cm Hg and then weighed in water again. They were released into aquaria, some with access while others were denied access by a stainless steel screen. Flotation pressures could thus be determined prior to and following pressure reduction and again after 2 wk either with or without access to the surface.

Extent of buoyancy adjustment — Year 0 tadpole madtom attained a significantly ($P < 0.05$) higher flotation pressure in both still water and current than did year 1 or 2 (Cochran's modified *t*-test). Within each age-group fish held in still water had a significantly higher flotation pressure than those in current although the difference decreased with age. Mean flotation pressures and numbers of fish (in parentheses) used in this analysis were as follows:

Yr	Length (*mm*)	Still	Current	Difference
0	28–36	1.098(8)	0.795(9)	0.303
1	50–65	0.903(7)	0.689(7)	0.214
2	75–90	0.861(11)	0.688(8)	0.173

Year 0 and 1 black bullhead in still water attained a significantly higher buoyancy than those in current while year 3+ did not make a significant adjustment. Too few year 2 fish were captured to permit analysis. In still water year 0 were significantly more buoyant than year 1 or 3+. Mean flotation pressures and numbers of fish (in parentheses) were as follows:

Yr	Length (*mm*)	Still	Current	Difference
0	25–34	1.000(8)	0.678(8)	0.322
1	60–80	0.858(15)	0.645(10)	0.213
3+	180–200	0.792(9)	0.781(8)	0.011

Saunders (1965) showed that after a week in still water brook trout (*Salvelinus fontinalis*) and Atlantic salmon (*Salmo salar*) parr had higher flotation pressures than those held in current. Similar results were obtained by Gee (1968; 1970; 1972) for longnose (*Rhinichthys cataractae*) and blacknose dace (*R. atratulus*). Smaller (year 0) tadpole madtom and black bullhead made greater adjustments of buoyancy than larger fish. A similar pattern was found for longnose dace although in blacknose dace the ability to adjust buoyancy increased with size (Gee 1972).

The range in buoyancy attained by tadpole madtom and black bullhead is considerably higher than that attained by stonecat, *Noturus flavus* (Machniak 1973). Such differences are adaptations to different environments. Tadpole madtom and black bullhead are typical of still to slow-moving waters (Trautman 1957; Taylor 1969) while stonecat are common in rapids of rivers and along wave-washed shores of Lake Erie (Taylor 1969). A similar relationship between buoyancy attained and environment occupied has been described for brook trout and Atlantic salmon parr (Saunders 1965) and species of the genus *Rhinichthys* (Gee 1972; Gibbons and Gee 1972).

Rate of buoyancy adjustment and varitaion in internal pressure — Tadpole madtom increased their buoyancy when water velocity changed from current to still (Fig. 1A). Most of the adjustment was made within 48 h and was completed in 96–192 h. Regression lines were calculated with a logarithmic transformation of time for rate of increase during the first 24 h. The regression lines were: $y = 0.838 + 0.071 \log x$ (year 0), $y = 0.795 + 0.056 \log x$ (year 1), and $y = 0.768 + 0.071 \log x$ (year 2). A test of homogeneity on regression coefficients showed no significant difference between rate of increase (slope) for the three age-groups.

When exposed to current most of the decrease in buoyancy occurred within 24–48 h with a complete adjustment requiring 96 h for year 0 and 192 h for older fish (Fig. 1B). Regression lines calculated for the rate of decrease in flotation pressure during the first 48 h were: $y = 1.033 - 0.117 \log x$ (year 0), $y = 0.940 - 0.044 \log x$ (year 1), and $y = 0.895 - 0.033 \log x$ (year 2). A significant difference ($P < 0.05$) between the slope of year 0 fish compared with each of the older groups was shown by a test of homogeneity on regression coefficients signifying a more rapid decrease in buoyancy by year 0 fish.

During the change from current to still water year 0 fish showed a decrease in internal pressure within 12 h while year 1 and 2 had less discernable changes (Fig. 1C). None of these differences within age groups were significant (from analysis of variance). During reduction of buoyancy years

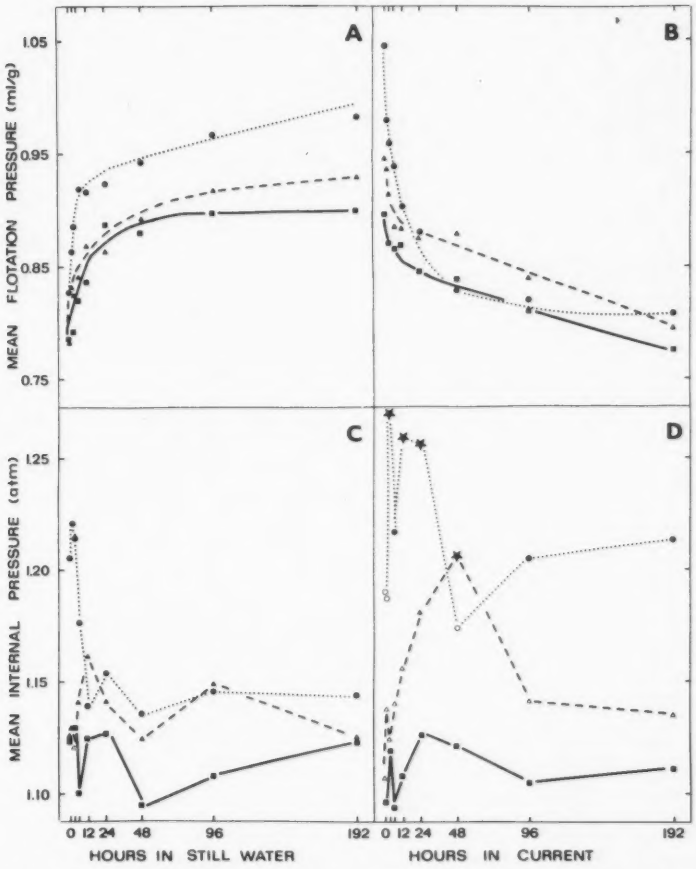

FIG. 1. Rate of adjustment of buoyancy (A, B) and changes in internal pressure (C, D) in tadpole madtom (n = 8). No observations were made at 1 h for year 2 fish. Circles = year 0; triangles = year 1; squares = year 2. Within each age-group means represented by stars differ significantly from open symbols.

0 and 1 fish increased internal pressure during the first 24 and 48 h, respectively. This was then followed by a decrease (Fig. 1D). Significant differences ($P < 0.05$) between means within age-groups were revealed by analysis of variance combined with Student-Newman-Kuels' test.

Rate of adjustment of buoyancy by tadpole madtom is considerably slower than for other species. Saunders (1965) showed that Atlantic salmon parr and brook trout completed adjustments within 48 h. Longnose and blacknose dace required 132 h (Gee 1968; 1970). These latter species made a quicker gain in buoyancy than a decrease as did tadpole madtom.

Excess internal pressure of swimbladder gases is common in Cypriniformes (Alexander 1959; 1961; Gee et al. 1974). Its alteration during adjustment of buoyancy could result from loss or addition of gases through the pneumatic duct, secretion or absorption of gases by the swimbladder wall, or compression or expansion of muscles lying in or about the swimbladder wall. During the decrease in buoyancy there was a significant increase followed by a decrease in

internal pressure in year 0 and year 1 tadpole madtom. This indicates that the initial reduction in swimbladder volume is brought about by a compression of swimbladder gases followed by either release of gases through the pneumatic duct or absorption of gases through the wall of the swimbladder. A number of studies have confirmed that changes in internal pressure are the result of muscular activity altering the volume of the swimbladder (McCutcheon 1958; 1962; Qutob 1962; Long 1959). Blacknose dace decreased internal pressure during increase in buoyancy and decreased internal pressure when buoyancy was reduced (Gee 1970). In both species there is considerable variation between fish in internal pressure, probably the rest of different rates at which fish adjust buoyancy.

Ability to secrete gases — Following a reduction of buoyancy tadpole madtom were able to increase flotation pressure significantly (Cochran's modified t-test; $P < 0.05$) only when they had access to the surface. However, black bullhead in access and non access were able to increase flotation pressure similar to that prior to buoyancy reduction. Mean flotation pressures and number of fishes examined (in parentheses) for A) tadpole madtom and B) black bullhead were as follows:

	Before buoyancy reduction	After buoyancy reduction	After 2 wk in still water; a) access	b) no access
A	0.956 (24)	0.840 (24)	0.967 (12)	0.843 (12)
B	0.903 (10)	0.751 (10)	0.909 (4)	0.923 (6)

This indicates that black bullhead can secrete gases readily to enlarge swimbladder volume. Tadpole madtom showed no such ability. Gulping air at the surface is their major means of increasing swimbladder volume. This does not preclude the possibility that tadpole madtom can secrete gases, possibly very slowly.

In non access, black bullhead were continually swimming about while tadpole madtom remained relatively inactive on the bottom. The inability of tadpole madtom to secrete that amount of gases to increase their buoyancy significantly might be due to this inactivity. Secretion of gases is greatest when fish are active (Jacobs 1930; Copeland 1952; McCutcheon 1962; Meesters and Nagel 1934). Fänge (1966) notes that fishes seem to secrete gases when they are "aware" that they are too heavy. Such an awareness could come about through motions of swimming. However, the greater activity of black bullhead may simply be the result of a quicker rate of gas secretion rather than the cause of such an effect.

The ability to adjust swimbladder volume to alter buoyancy is an important adaptation assisting fishes to survive in a lotic environment where water velocity varies considerably.

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Atlantic Salmon (*Salmo salar*) Taken in the Sea in North Labrador

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POWER, G., AND D. CRESSMAN. 1975. Atlantic salmon (*Salmo salar*) taken in the sea in north Labrador. J. Fish. Res. Board Can. 32: 307–309.

Scale analysis showed that with few exceptions salmon taken near the northern extremity of Labrador were similar to stocks from Ungava rivers; those taken near Nain were probably of local origin. Knowledge of the distribution of local stocks of salmon in the sea contributes to our understanding of their migrations and is valuable in view of the recent development of commercial fisheries for this species in the northwest Atlantic.

POWER, G., AND D. CRESSMAN. 1975. Atlantic salmon (*Salmo salar*) taken in the sea in north Labrador. J. Fish. Res. Board Can. 32: 307–309.

Les saumons capturés près de la pointe nord du Labrador sont semblables aux stocks des rivières de l'Ungava; ceux qui sont capturés près de Nain sont probablement d'origine locale. L'examen des écailles démontre que quelques saumons originaires des rivières situées au sud peuvent s'approcher de la côte et être capturés avec les stocks des rivières du nord. De récents retours d'étiquettes viennent d'ailleurs appuyer cette conclusion. Ces résultats présentent un intérêt particulier en ce qui concerne le développement des pêches commerciales du saumon dans le nord-ouest de l'Atlantique.

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THIS note provides details of the weights, lengths, and ages of salmon taken in the sea in the vicinity of Port Burwell and Nain (Fig. 1). Ages and scale structure indicate the fish are of local origin which is significant in relation to the recent development of commercial fisheries for this species in the northwest Atlantic.

The appearance of salmon near the north Labrador coast is not new; the Hudson's Bay Company exported five barrels (1515 lb) from Port Burwell as early as 1921 (Housing Account, St. Olave's and Pickle Herring Wharves, Tooly St., London, 27/9/1921). Recently the Federal Department of Northern and Indian Affairs has encouraged cod and Arctic char fishing in this vicinity and some salmon were taken. Fishing is carried out both on the Ungava Bay and the Atlantic side of the northern tip of Labrador and in the connecting straits. Salmon catches have fluctuated from a high of 250 fish in 1967 to a

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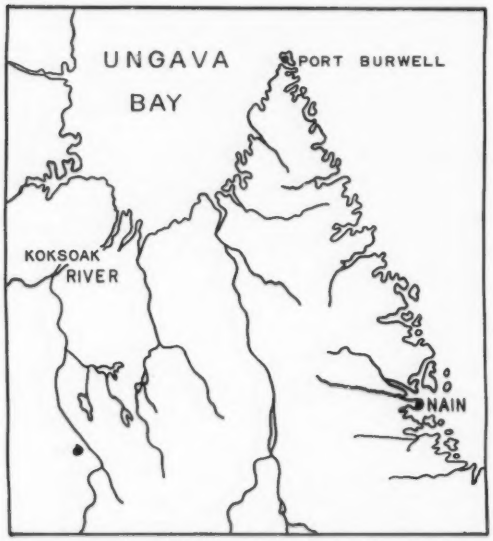

FIG. 1. North Labrador and Ungava Bay showing locations where salmon samples were obtained.

low of 9 in 1972 (R. B. Tinling personal communication). The salmon are usually gilled in the 4-inch (102-mm) stretched mesh leaders of codtraps or taken in 5.5-inch (140-mm) stretched mesh gillnets set for Arctic char.

Farther south more salmon are taken. Recent landings at Nain, the most northerly point recording commercial catches, were 1970, 22,313 lb; 1971, 32,816 lb; and 1972, 17,248 lb (W. H. Lear personal communication).

In 1959 Mr. M. Tiller provided scale samples from 36 salmon taken in codtraps set from offshore islands near Nain. Weights are unknown and lengths are approximate as the fish had been salted when measured. In 1967 sixty-eight salmon taken close to Port Burwell and six taken nearby on the Atlantic coast (Jackson Island, Taliavsik and Eclipse Channel) were examined. All were classed as Burwell fish as there was nothing to suggest the Atlantic coast fish should be treated separately.

The Nain fish were predominantly small with a high proportion of grilse (Table 1). Many had spent 5 yr or more as juveniles in fresh water (Table 2). The duration of their freshwater life is almost 1 yr greater than that recorded for the Sandhill River in southern Labrador (Peet and Pratt 1972) or for the Labrador commercial catches (predominantly fish taken in southern Labrador) by Lear and May (1972). The high river age of the Nain fish suggests they were taken as they approached the coast to ascend local rivers in which the duration of river life can be assumed to be longer than in rivers farther south.

The Burwell fish were very like Ungava stocks and indeed the majority of them may have been returning to this area to spawn. Assuming that Ungava salmon return to their river of origin to spawn, evidence of long migration was provided by a salmon that was marked on October 2, 1970 at Kigdlut (63°63′N, 51°22′W) near Godthaab in West Greenland and recaptured in the Koksoak River on August 30, 1971. It had spent 5 yr in fresh water before going to sea (W. Shearer personal communication). The majority of Koksoak fish spend 5 yr in fresh water before seaward migration (Table 2) and indirect evidence from sea growth shows that they grow as rapidly as salmon from more southerly rivers, suggesting they share common feeding areas in the sea (Power 1969).

The smaller average size of Burwell fish, compared to Koksoak River fish (Table 1), is possibly a result of selectivity of fishing gear. A 4-inch mesh codtrap leader will select smaller fish than a 5.5-inch mesh salmon net. The Burwell fish were taken between September 4 and 28, while landings in the Koksoak indicated the major run was over in that river before the end of August when commercial fishing stopped. The partly recovered kelts taken at Burwell were larger and heavier than those from the Koksoak. Assuming they were from Ungava rivers, the difference in size can be explained on the basis of the Burwell sample of mended kelts having had an extra month feeding at sea.

The scale structure and river age of some of the Burwell fish indicates they are unlikely to be of Ungava origin. Five of the 56 2+ maiden salmon taken at Burwell spent 3 yr in fresh water, whereas in the years 1966–67 in a sample of 2428 2+ maiden salmon entering the Koksoak and Whale rivers, Ungava, only three spent as little as 3 yr in fresh water. Ungava salmon scales can be recognized by their clearly defined annuli and by the large size of the central area developed during river life. This is a reflection of the large size of Ungava salmon smolts when they enter the sea (Power 1969). The 3-river-year fish in the Burwell sample had small central areas on their scales typical of salmon that had grown in rivers where smolt size was much less.

Recently a number of tags applied to salmon in Maine and the Maritimes have been recovered in Labrador. These include five smolts and an adult released in the Restigouche (C. P. Ruggles

TABLE 1. Numbers and fork lengths (FL) of the predominant classes sampled from Nain (1959), Port Burwell (1967), and the Koksoak River (1967).

Class of salmon	Nain		Port Burwell		Koksoak River	
	No.	FL (*cm*)	No.	FL (*cm*)	No.	FL (*cm*)
Grilse	16	52.9	8	58.4	274	63.9
2+ maiden	7	72.9	56	74.9	820	82.2
Kelts	10	58.8	8	79.6	456	76.4
All others	3	55.0	2	68.9	143	73.0

TABLE 2. The age of grilse and 2+ maiden salmon taken in the sea near Nain (1959) and Port Burwell (1967) and in the estuary of the Koksoak River (1967). The last year's growth, which is almost complete, is counted as a full year; i.e. a 3.2+ salmon is 6 yr old.

Class of salmon	Location	Age in years				
		6	7	8	9	10
Grilse	Nain	4	8	3	1	–
	Burwell	3	2	3	–	–
	Koksoak	18	186	67	3	–
2+ maiden	Nain	1	2	1	2	1
	Burwell	5	20	26	3	2
	Koksoak	–	156	550	105	9

personal communication) and 42 recoveries from salmon tagged in Maine (A. L. Meister personal communication). The majority of these tags were returned from Nain southwards but one Maine fish was caught in Eclipse Channel, which supports the deduction from scale reading that a few southern fish may be intermingled with stocks of local origin taken near the tip of Labrador.

Interest in salmon taken in the sea in the northwest Atlantic area has been prompted by the development of the west Greenland coastal fishery and the offshore fishery for Atlantic salmon in Davis Strait (Saunders et al. 1965). Salmon can also be taken on the Labrador side of Davis Strait and it is possible they approach the coast of southeastern Baffin Island which is not very far from the tip of Labrador. The samples described here from Port Burwell and Nain appear to be mostly of local origin. It would be interesting to know more about these fish, the times of year they approach the coast, and the pattern of their movements.

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DDT Residues in Euphausiids in the Upper Estuary of the Gulf of St. Lawrence

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SAMEOTO, D. D., D. C. DARROW, AND S. GUILDFORD. 1975. DDT residues in euphausiids in the upper estuary of the Gulf of St. Lawrence. J. Fish. Res. Board Can. 32: 310–314.

No significant variation was found in organochlorine residue levels between different stations in an area from the Saguenay River to the end of the Gaspé Peninsula in the Gulf of St. Lawrence for the euphausiids *Thysanöessa raschii*, *T. inermis*, and *Meganyctiphanes norvegica*. Significant differences were found between the concentration of organochlorine residues for *M. norvegica* and the *Thysanöessa* species, but there was no difference between the two *Thysanöessa* species.

Significantly lower values for percent lipid were found in *T. inermis* and *T. raschii* between June 1973 and May 1974 samples, but the total amount of organochlorine residues did not change. This suggests that these species do not lose *p,p'*-DDT when they metabolize lipids for energy or the production of gonad material.

SAMEOTO, D. D., D. C. DARROW, AND S. GUILDFORD. 1975. DDT residues in euphausiids in the upper estuary of the Gulf of St. Lawrence. J. Fish. Res. Board Can. 32: 310–314.

Nous n'avons pas trouvé de différence significative de niveau de résidus organochlorés chez les euphausides *Thysanöessa raschii*, *T. inermis* et *Meganyctiphanes norvegica* à différentes stations échelonnées depuis le Saguenay jusqu'à l'extrémité de la péninsule de Gaspé, dans le golfe Saint-Laurent. Nouse avons observé des différences significatives de concentration de résidus organochlorés entre *M. norvegica* et les espèces de *Thysanöessa*, mais il n'y a pas de différence entre les deux espèces de *Thysanöessa*.

Nous avons observé une diminution significative du pourcentage des lipides de *T. inermis* et *T. raschii* entre les échantillons de juin 1973 et de mai 1974, mais la quantité totale de résidus organochlorés est demeurée la même. Ceci donne à croire que ces espèces ne perdent pas de *p,p'*-DDT lorsqu'elles utilisent leurs lipides pour la production d'énergie ou de matériel gonadique.

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THE large concentrations of euphausiids present in the Gulf of St. Lawrence (Sameoto 1972) are not only important components of the marine food web, but are a possible human food resource. Therefore it is important to know the level of potentially harmful contaminants present in the animals. In this study, we report concentrations of DDT[1] group residues found in samples of three of the commonest species, *Meganyctiphanes norvegica*, *Thysanöessa raschii* and *T. inermis* collected in the spring of 1973 and 1974.

Methods — The following abbreviations are used: *p,p'*-DDT, 2,2-bis-*p*-chlorophenyl-1,1,1-trichloroethane; *p,p'*-DDE, 2,2-bis-*p*-chlorophenyl-1,1-di-chloroethylene; *p,p'*-DDD, 2,2-bis-*p*-chlorophenyl-1,1-di-chloroethane.

Samples of euphausiids were taken during June 1973 and May 1974 at the sattions shown in Fig. 1 with a 0.7-mm mesh, ¾-m monofilament plankton net. The specimens of all three species had a similar age and size structure in both years. Two additional samples of *M. norvegica* were taken off the coast of Nova Scotia near Canso on April 10, 1973. The animals were identified and separated manually from other forms of zooplankton, rinsed with clean sea water, and then frozen at –20 C until analysis.

Organochlorine pesticide residues and lipids were extracted from duplicate 10-g samples of euphausiids by homogenizing with chloroform–methanol (Folch et al. 1957). Duplicate extractions were made where samples were sufficiently large. All solvents were

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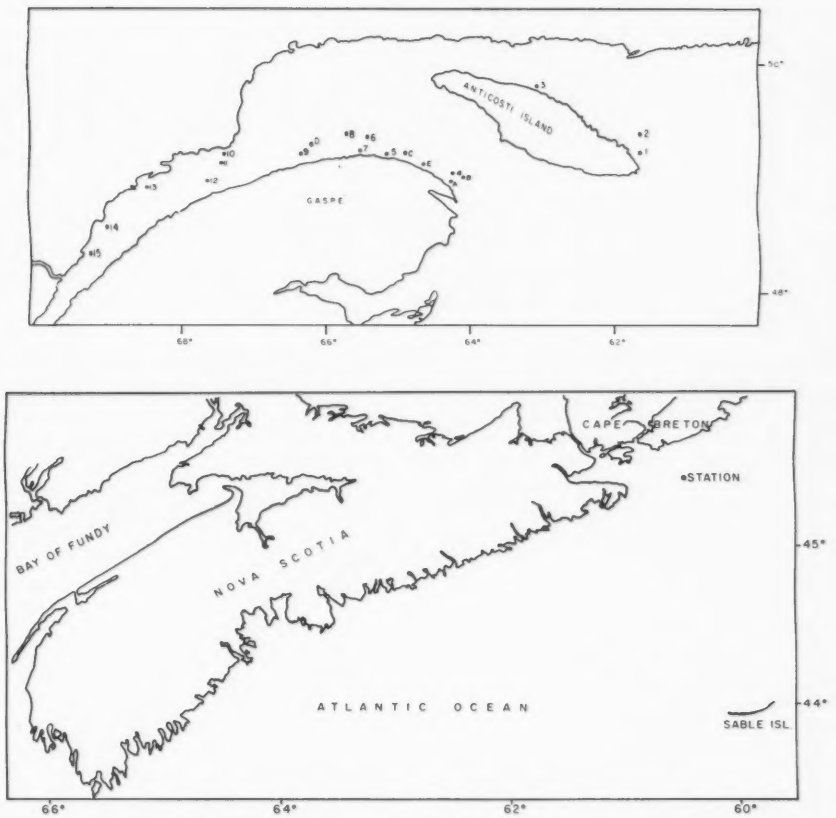

FIG. 1. Locations of the sampling stations in the Gulf of St. Lawrence (*upper map*) and off the coast of Nova Scotia.

reagent grade, redistilled from glass. (Although chloroform-based extractants are not widely used in chlorinated hydrocarbon analysis, they have been used successfully for samples not amenable to complete extraction by other procedures: e.g. Radomski and Rey 1969; Versino et al. 1971.) Additional representative samples were "spiked" before extraction by injecting into the animals a 2–5 μl acetone solution of ^{3}H-*p,p'*DDT (New England Nuclear Corp.) as an internal standard for recovery determinations. The chloroform phases of the extracts were reduced to a small volume by gentle rotary evaporation, taken up in a few milliliters of hexane, and reduced again to a small volume; this procedure effectively removed all chloroform from the extracts. Aliquots of the final hexane solution were taken for gravimetric lipid determination, cleaned up by chromatography on Florisil, and analyzed by gas–liquid chromatography (GLC) essentially as described by Addison et al. (1972). At least three replicate determinations were made on each sample. Single label liquid scintillation counting was performed basically as described by Darrow and Addison (1973). Following a preliminary estimate of DDT group levels, samples were routinely dehydrochlorinated (Holden and Marsden 1967) for confirmation of *p,p'*-DDT through conversion to *p,p'*-DDE.

Results and discussion — Mean recovery of ^{3}H-*p,p'*-DDT through extraction and column chromatography was 83 ± 11% (n = 15) and all data have been corrected to a 100% recovery. Results are presented in Table 1. Lipid content varied from 0.96 to 10.15% wet weight, depending on the species and time of the year. Residue concentrations are expressed as ppb wet weight and ppb total lipid. Only residues of *p,p'*-DDE and *p,p'*-DDT were confidently detected in *M. norvegica* and *T. inermis*; in *T. raschii* an overlapping peak in the 1973 samples prevented

TABLE 1. Organochlorine residue concentrations and lipid content of euphausiids in Gulf of St. Lawrence. Each hexane determination is the mean of at least three replicate analyses of one or more samples presented as ppb of wet weight and lipid weight. Lipid concentration (of single or duplicate samples) is expressed as percentage wet weight.

			Wet wt		Lipid		
Date	Species	Station	*p,p'*-DDE	*p,p'*-DDT	*p,p'*-DDE	*p,p'*-DDT	% lipid
June 73	*Meganyctiphanes norvegica*	4	8.9±2.7	16.2±4.9	331±114	598±185	2.73±0.15
		7	7.0±1.2	19.0±6.0	245±36	673±199	2.84±0.06
		8	13.1±2.5	40.7±7.1	357±44	1187±161	3.55±0.17
		9	10.0±1.8	38.3±4.1	460±76	1757±160	2.18±0.06
		12	8.5±2.8	26.6±8.9	475±171	1485±431	1.80±0.11
		13	23.0±3.0	41.8±3.2	937±47	1718±128	2.49±0.34
		15	10.6±1.8	18.5±4.5	518±93	889±201	2.07±0.05
Apr. 73		Nova Scotia coast	6.9±1.9	21.9±4.4	310±77	1002±216	2.22±0.22
			8.1±1.4	24.2±1.8	352±65	1073±135	2.27±0.07
May 74		C	11.3±1.2	21.7±1.1	393±32	760±59	2.27±0.25
		B	7.7±1.5	17.9±3.6	334±59	753±148	2.39±0.23
		E	11.8±1.6	20.3±6.7	495±27	933±169	2.88±0.12
June 73	*Thysanöessa raschii*	6	n.d.[a]	17.1±3.9	n.d.	704±158	2.43±0.01
		10	n.d.	14.3±1.1	n.d.	685±79	2.09
		11	n.d.	13.0±2.3	n.d.	323±81	4.02
		11	n.d.	6.9±0.9	n.d.	419±64	1.65±0.11
May 74		D	4.0±0.7	18.0±1.8	410±79	1047±161	0.96±0.04
		A	4.9±0.6	10.9±1.8	348±60	777±114	0.98±0.20
		C	3.6±0.1	7.1±0.6	367±54	779±118	1.47±0.17
		F	2.0±0.3	6.7±0.5	114±19	377±38	1.77±0.05
June 73	*T. inermis*	1	4.7±1.5	7.7±0.9	58±15	95±11	8.10
		2	2.7±0.5	7.0±0.9	24±4.7	68±8.6	10.15±0.21
		4	n.d.	9.4±3.7	n.d.	122±56	7.66±0.67
		5	3.5±0.7	6.0±0.4	53±10	93±5.4	6.44±0.02
May 74		A	4.0±0.3	6.8±0.4	417±10	704±68	0.98±0.08
		D	2.9±0.2	5.6±0.6	178±8.2	341±38	1.63

[a]n.d. – not determined.

reliable quantification of *p,p'*-DDE. A peak co-chromatographing with *p,p'*-DDD was observed in all samples, but was too small to be measured reliably. ΣDDT (*p,p'*-DDE + *p,p'*-DDT) ranged from approximately 900 to 2700 ppb lipid in *M. norvegica*, 300 to 1000 ppb lipid in *T. raschii*, and 100 to 1100 ppb lipid in *T. inermis*. Duplicate analysis agreed to within 20% at residue concentrations of approximately 500 ppb lipid (minimum detectable concentration of the DDT group was approximately 50 ppb lipid).

p,p'-DDT levels in spiked samples were higher than those in the unspiked replicates, but the difference between total ("hot" plus "cold") DDT less the "hot" DDT as determined by radioassay was similar to the level of "cold" DDT in the unspiked sample.

Peaks with retention times similar to those in polychlorinated biphenyl (PCB) standard (Aroclor® 1254) were found in all dehydrochlorinated samples, but relative peak height ratios differed too much between sample and standard to allow reliable quantification.

There was no significant variation in residue levels with sample stations among the three species. Levels of organochlorine residue in *M. norvegica* from the Scotian Shelf were similar to the 60 ppb (wet weight) of the DDT group found in *M. norvegica* taken from the stomach of a female fin whale off Nova Scotia in November 1967 (Addison et al. 1972) and were not significantly different ($P < 0.05$ by t test) from those found in the animals in the Gulf. This is consistent with the view that the main delivery path for DDT to the ocean is the atmosphere (Seba and Prospero 1971) and so large spatial variation would not be expected.

Differences in concentration of organochlorine residues for both wet weight and lipid weight between *M. norvegica* and *Thysanöessa* spp. were shown to be significant by an analysis of variance ($F = 16.63$, df = 2,16, for wet weight; $F = 16.05$, df = 2,16, for lipid weight). The means for the three species were tested for significant differences by a modified Duncan's multiple range test (Kramer 1956). The results showed a significant difference between *M. norvegica* and the two species of *Thysanöessa*, but there was no significant difference between the *Thysanöessa* species. Since *M. norvegica* is mainly carnivorous, feeding on copepods, while *T. raschii* and *T. inermis* are mainly phytoplankton feeders, the difference might indicate selective accumulation at higher trophic levels. However, such processes may not be the primary mechanism of residue uptake at the upper trophic levels (Hamelink et al. 1971; Risebrough et al. 1972). Alternatively, such differences may reflect differing abilities of the species to selectively excrete or degrade certain compounds. Variation in the lipid composition of the species examined (Mauchline and Fisher 1969) may also affect their ability to retain chlorinated hydrocarbons.

There were significant differences in the percent lipid for *T. inermis* and *T. raschii* between the May 1974 and June 1973 samples ($F = 53.21$, df = 1,8; $P < 0.01$; $F = 9.93$, df = 1,8; $P < 0.01$). There was no significant difference between years for *M. norvegica*. The amount of *p,p'*-DDE and *p,p'*-DDT per unit wet weight was not significantly different between years for any of the three species. But *T. inermis* had significantly more *p,p'*-DDT expressed in terms of lipid weight in May 1974 than in June 1973, even though the total amount of *p,p'*-DDT did not change in the whole animal from 1 yr to the next. This suggests that *T. inermis* does not lose *p,p'*-DDT when it metabolizes lipids for energy or for the production of gonad material. *Thysanöessa inermis* and *T. raschii* both spawn in April and early May in the Gulf (Berkes 1973), which may account for the lower lipid values in May.

In general, residue levels in euphausiids were similar to those found in other forms of marine zooplankton (Risebrough et al. 1972; Kerr and Vass 1973) and were generally below those reported calculated for various marine organisms from the same area (Sprague and Duffy 1971).

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An Automatic Brine Shrimp Feeder for Aquatic Bioassays[1]

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SCHIMMEL, S. C., AND D. J. HANSEN. 1975. An automatic brine shrimp feeder for aquatic bioassays. J. Fish. Res. Board Can. 32: 314–316.

An electrically operated brine shrimp feeder is described. The device may be set to cycle 1–12 times each day for tests in fish and invertebrate culture and bioassay. Major advantages of the feeder are that it is readily adapted to flow-through bioassay and culture apparatuses which require that equal quantities of food be delivered to animals in two or more test aquaria and that the number of feedings be recorded. The components, all readily available, cost approximately $190.

SCHIMMEL, S. C., AND D. J. HANSEN. 1975. An automatic brine shrimp feeder for aquatic bioassays. J. Fish. Res. Board Can. 32: 314–316.

Les auteurs décrivent un distributeur d'artémies actionné à l'électricité. L'appareil peut être réglé selon un cycle de 1–12 fois par jour et servir à nourrir des poissons et des invertébrés lors d'essais de culture ou d'analyse biologique. Il a comme avantages principaux d'être facilement adaptable à des appareils d'analyse biologique ou de culture traversés par un débit d'eau, de pouvoir donner aux animaux maintenus dans deux ou plusieurs aquariums une même quantité de nourriture et d'enrégistrer le nombre de repas. Les composantes, toutes faciles à trouver, coûtent environ $190.

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IN this report, we describe an automatic brine shrimp feeder that is more useful than others (Benoit et al. 1969; Anderson and Smith 1971) in that it delivers equal quantities of food to culture or bioassay tanks and records the number of feedings.

The feeder (Fig. 1) consists of a compartmented, all-glass container, two 115-V Gems®

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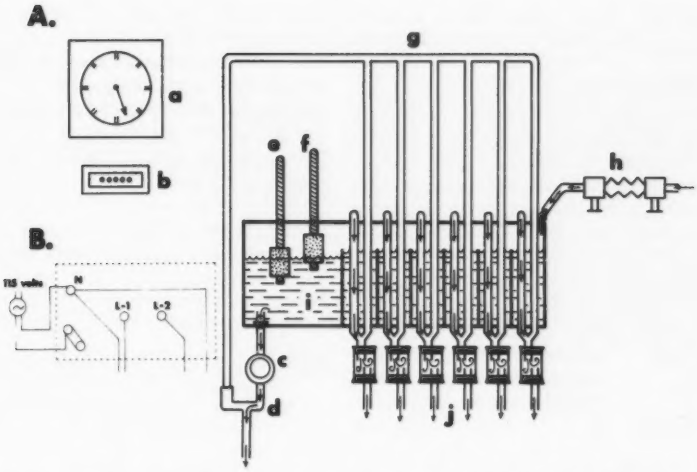

FIG. 1. Automatic brine shrimp feeder. A. a, electric timer; b, electric counter; c, solenoid valve; d, vacuum venturi; e and f, float switches; g, vacuum manifold; h, oscillating pump; i, float switch compartment; j, siphon tubes to aquaria. B. Timer schematic. L-1 and N, circuit to float switch f and to oscillating pump (h); L-2 and N, circuit to float switch e, solenoid valve (c), and counter (b).

float switch systems, an oscillating pump, a stainless steel solenoid valve, and an electrical counter and Intermatic® timing mechanism. The glass container, 32 × 14 × 4 cm, is divided into seven compartments, six of which hold 100 ml each. The six compartments provide equal numbers of nauplii to culture or bioassay aquaria (compartment i may also be used if carefully calibrated). Although we selected the compartment number and size to fit our needs, the dimensions may be varied to suit other requirements.

The brine shrimp are incubated and hatched in aerated, 2-liter separatory funnels apart from the feeder (24–48 h at room temperature). The nauplii are separated from the eggs by shutting of air supply and allowing differential settling. Nauplii are placed in a reservoir (approximately 20–30 liters of 1% salt solution, depending on volume and rate of feedings). The reservoir (not illustrated) is also aerated to provide oxygen and disperse the animals uniformly throughout the seawater medium. The nauplii remain in the reservoir until pumped out at intervals dictated by settings on the timer. In tests, nauplii remained in the reservoir for up to 4 days without appreciable mortality or bacterial contamination. Using the feeder and reservoir volumes we describe, the reservoir should be refilled every 48 h. Population density of brine shrimp may be calibrated by a photometric device or Sedgwick–Rafter counting cell to ensure uniform density when refilling the reservoir.

The operating sequence is: the timer mechanism (a), through one of twelve possible "trippers" that cover a 24-h period, energizes the oscillating pump (h) and float switch f through electrical connection L-1 (Fig. 1B). The pump fills each compartment with nauplii and water from the reservoir; the mixture finally cascades into the largest compartment (i), raising float switches e and f. On rising, float switch f opens, deactivating the oscillating pump, while float switch e closes. The closing of switch e does not cause the feeder to empty until the timer's tripper mechanism releases (duration can be adjusted from 5 to 60 min). When the tripper mechanism releases, circuit L-2 is energized and activates the solenoid valve (c) and counter (b) through float switch e. The purpose of the counter is to number the feedings, since the device is updated each cycle. The solenoid valve now opens, allowing water in compartment i to flow, creating a vacuum at the venturi d. The vacuum, applied along the entire manifold (g), starts the siphons in the small compartments. The nauplii in these compartments are channelled to culture or bioassay aquaria through siphon tubes (j). As the largest compartment (i) empties, float switch e

opens and switch f closes. The system is now ready to recycle when the timing mechanism trips again.

Our feeder has been used (Schimmel et al. 1974) in several 30-day bioassays utilizing a modified Mount–Brungs diluter. Since the total daily volume from the feeder was 600 ml and the daily diluter volume was 40 liters, concentration of toxicant in these bioassays was not appreciably affected (note: feeder siphons empty directly into diluter delivery tubes but the connections must not be airtight as the two devices cycle independently). During our bioassays no mechanical or electrical difficulties occurred. If freshwater cultures or bioassay experiments are attempted, salt content of the reservoir should not be a problem, provided a sufficient ratio of freshwater volume to feeding volume is used. Reservoir water of 1% salt solution will easily support brine shrimp cultures.

Cost of the individual components of our feeder is approximately $190, and the items described here are available in most electronic catalogs.

Acknowledgments — We thank Mr S. Foss for preparing Fig. 1.

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Differentiation Between Natural Hydrocarbons and Low Level Diesel Oil Contamination in Cooked Lobster Meat

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PARADIS, M., AND R. G. ACKMAN. 1975. Differentiation between natural hydrocarbons and low level diesel oil contamination in cooked lobster meat. J. Fish. Res. Board Can. 32: 316–320.

A combination of total lipid extraction, column chromatography, and temperature-programmed gas chromatography was required to demonstrate disputed low level diesel oil contamination in cooked lobster meat. A specific diesel oil contaminant was indicated but identifiable components were not greatly in excess of the same compounds which were also found to be a normal background in organoleptically acceptable canned lobster meat.

PARADIS, M., AND R. G. ACKMAN. 1975. Differentiation between natural hydrocarbons and low level diesel oil contamination in cooked lobster meat. J. Fish. Res. Board Can. 32: 316–320.

A small-scale spill in the harbor of Souris, Prince Edward Island, resulted in the exposure of lobsters in holding crates to diesel oil for 12–24 h. The crates showed visual evidence of fouling with diesel oil. The lobsters were removed to a clean area of the harbor and held for 5–7 days, then canned experimentally, and finally canned "en masse." Separate taste panel tests with the cooked lobster meat were conducted by the canner and by the Fisheries Inspection Laboratory, Halifax, N.S. The tests gave conflicting results and a chemical analysis was requested of this labora-

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tory. A simple method based on steam distillation and isothermal gas chromatography (Ackman and Noble 1973), previously used to examine tainted fish, met with limited success. Only traces of hydrocarbon appeared in the steam distillate, possibly due to prior removal of shorter chain-length hydrocarbons during lobster processing by steaming or boiling. An alternative method (Mackie et al. 1972) involving extraction, column chromatography, and temperature-programmed gas chromatography was then used. This second method allowed the concentration of large amounts (several hundred micrograms) of hydrocarbons from large amounts of cooked meat. The hydrocarbons obtained from control and "polluted" meat were compared to several commercial diesel oils. A specific source of contamination was strongly indicated.

Samples — All lobster meat samples were supplied to us by the Fisheries Inspection Laboratory, Halifax, N.S., and were prepared commercially as cooked, frozen, lobster meat in 12-oz cans. The meat was kept canned and frozen until analyzed.

Isolation of hydrocarbons — Attempts to examine the lobster meat by steam distillation were carried out in the same manner as that previously described by Ackman and Noble (1973). The low level of contamination required an alternative large-scale method of extraction and column chromatography which was carried out as follows: lobster meat was extracted with solvents redistilled in glass by the method of Bligh and Dyer (1959). The chloroform extract of total lipid was concentrated to a small volume in a rotary evaporator, with water at 20 C in the bath, under moderate water aspirator vacuum. Care was exercised not to eliminate all liquid at any time. The concentrated lipid solution was applied to a column 2.5 cm ID × 30 cm in length packed with a mixture of two parts silicic acid (100 mesh, Mallinckrodt) and one part Dicalite 4200 (Great Lakes Carbon Corp.). These were used as received (not activated) but were prewashed in, and the column equilibrated with, freshly distilled hexane. Columns were loaded with no more than 3 g lipid/100 g column packing. The hexane eluates were collected in 50-ml portions. The first 150 ml yielded a clean hydrocarbon fraction which was shown by thin-layer chromatography (TLC) to be free of any other lipid components. The hydrocarbon fraction was associated with a pale yellow pigment (possibly an isoprenoid hydrocarbon such as squalene) which simplified identification of the appropriate fraction during chromatography. All hydrocarbon fractions were nevertheless examined by thin-layer chromatography on Kodak Chromagram silica gel sheets, solvent n-hexane:diethylether:acetic-acid::85:15:1, before pooling for analysis by gas chromatography.

Gas chromatography — All analyses were carried out on Perkin-Elmer Model 900 gas chromatography equipment with flame-ionization detectors, fitted with wall-coated open-tubular stainless steel columns (150 ft in length × 0.01 inch ID) using: (i) a coating of Apiezon-L (AP-L), and temperature programming from 60 to 220 C at a rate of 5 C/min, or (ii) a coating of butanediolsuccinate (BDS) polyester, with temperature programming from 120 to 170 C at a rate of 5 C/min. In both cases He (60 psig) was used as the carrier gas. Programming was initiated on elution of the solvent and the final temperature was maintained for 60 min. The samples were concentrated when necessary (under N_2 at room temperature) to give adequate response and minimal baseline rise from programming during gas-chromatographic analyses.

Results and discussion — Analysis of three samples of commercial marine diesel oils (shown in part in Fig. 1) showed a distribution of straight-chain hydrocarbons from C_9 to C_{24} superimposed on a broad "envelope" region of unresolved minor peaks. Pristane (2,6,10,14-tetramethylpentadecane) and phytane (3,7,11,15-tetramethylhexadecane) occur as relatively large peaks, reasonably resolved from adjacent n-C_{17} and n-C_{18} hydrocarbons. Components with well-defined peaks were identified by the use of mixed runs with standard hydrocarbons. These chromatograms and the isomer ratios (Table 1) are characteristic of marine diesel oil and supply sufficient information to suggest the general origin of the crude from which two of the oils were derived as oil A — Middle-East crude; oil B — Venezuelan crude (Zafiriou et al. 1972). Oil C may be a mixture of fuel oils or may have been refined from a mixture of crudes.

These chromatograms also confirm the advisability of using temperature-programmed gas chromatography rather than low-temperature isothermal gas chromatography for diesel oil analysis, since the latter method examines only the first 10–15% of the hydrocarbon spectrum (C_9–C_{15}) which does not include the very characteristic C_{16}–C_{20} region.

Procedural blanks and experiments with standard hydrocarbons indicated that losses due to evaporation of hydrocarbons of molecular weight greater than C_{15} were very small or negligible if temperatures were maintained at, or below, room temperature and if evaporation to dryness was avoided. Results from the steam distillation method are not included here. Yields were not sufficiently high to permit adequate identification of the very long-chain hydrocarbons. No convenient way was found to increase the scale of experimental design which should, however, be adequate

TABLE 1. Isomer ratios for commercially obtained marine diesel oils, "polluted" and control lobster meat. Only peak heights (including the "envelope") are considered in calculating the ratios (Zafiriou et al. 1972).

Sample	Ratios			
	n-C_{17}:pristane	n-C_{18}:phytane	pristane:phytane	n-C_{17}:n-C_{18}
Marine diesel oil A	2.65	2.08	0.89	1.13
Marine diesel oil B	1.78	1.64	1.03	1.12
Marine diesel oil C	1.28	2.04	1.51	0.95
"Polluted" lobster meat[a]	1.26	1.84	1.60	1.10
Control lobster meat[a]	0.14	3.47	22.70	0.90

[a]These samples required the application of minor correction factors due to the use of much lower attenuations.

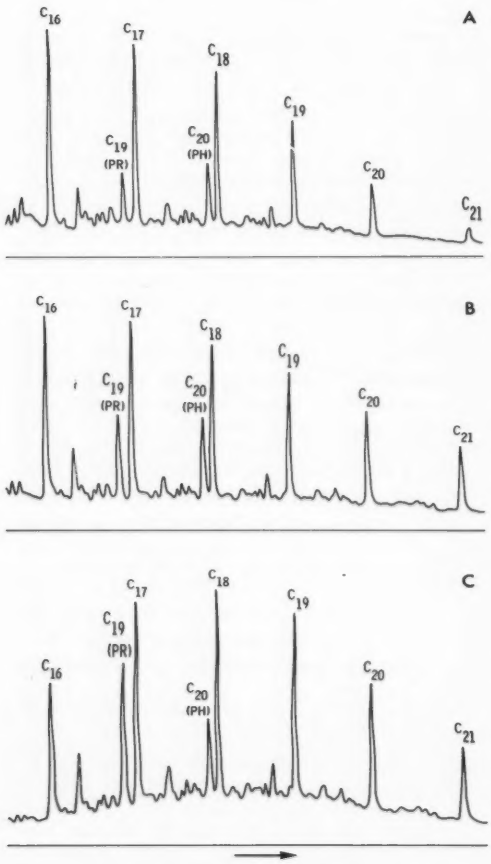

FIG. 1. Comparisons of C_{15}–C_{21} range of gas chromatograms of three different commercial diesel oils (A–C). All were run at the same attenuation (64×). The baseline shown is recorder zero; the rise in the pen trace is from this level. Column, Apiezon-L.

for higher concentrations of volatile hydrocarbon oils in fatty tissues. A chromatogram of hydrocarbons from one control sample is shown in Fig. 2 and sample isomer ratios are presented in Table 1.

In all, five control (or commercially acceptable) lobster meats were examined by the extraction-column chromatography method. All five contained very small amounts of hydrocarbon (2–4 ppm). All of these samples were characterized by high levels of n-C_{17} and pristane superimposed on a background similar to that of marine diesel oil. Motohiro and Inoue (1973) observed proportions of pristane and n-C_{17} similar to those in Fig. 2 in muscle of chum salmon (*Oncorhynchus keta*) contaminated with crude oil. Several unidentified components were found in all lobster meat hydrocarbon preparations, but did not seriously hamper the analyses. These compounds were not straight chain methyl esters of fatty acids, fatty aldehydes, or methyl ketones and were quantitatively more significant relative to hydrocarbons at the analytical levels employed in control meat and steam distillates than in unaccepable lobster meat and in lipid extracts.

The hydrocarbon findings suggest that even organoleptically acceptable lobster contains an appreciable background of extractable volatiles upon which a natural hydrocarbon pattern, high in pristane, n-C_{17}, and smaller amounts of other normal hydrocarbons, is superimposed. This interpretation is supported by the findings of other researchers who have shown that pristane and n-C_{17} or other straight-chain hydrocarbons, and some monomethyl hydrocarbons, are common in marine plankton and algae (Clark and Blumer, 1967; Blumer et al. 1971; Youngblood et al. 1973). Pristane is found in lipids of a variety of marine animals and often in large amounts in planktonic crustaceans (Blumer et al. 1964; Ackman 1971; Inoue et al. 1973). It has been

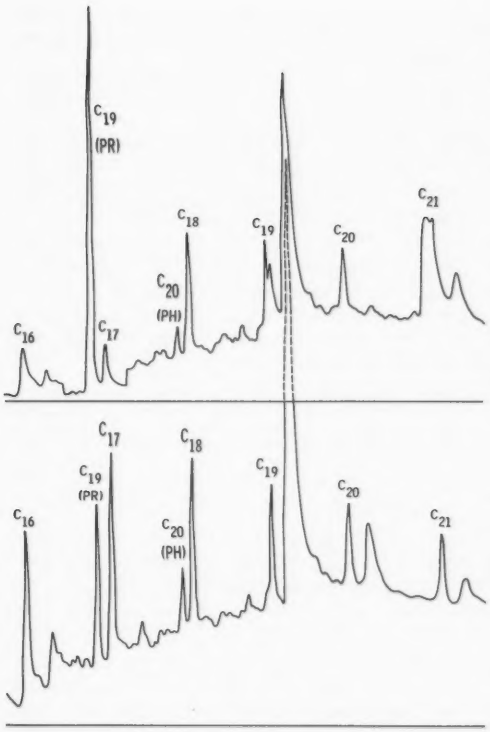

FIG. 2. Comparisons between C_{15}–C_{21} range of gas chromatogram of a typical "control" lobster meat (*upper*) and a suspect (polluted) sample (*lower*). Control run at 8× attenuation except for pristane and C_{17} peaks which were attenuated to 16×. Suspect sample run at 4×. Column, Apiezon-L.

definitely identified in lobster lipids, but in unspecified amounts (Blumer et al. 1964). Pristane is a major component of the mixture of hydrocarbons in the hepatopancreas of the crabs *Chionoecetes opilio, Lithodes turritus,* and *Paralithodes camtschatica* (Kawada et al. 1973). There is thus good reason to consider it a normal if minor component of the lipids of most larger marine crustacea.

The hydrocarbon "envelope" on the other hand, occurs primarily in oils or crudes. Phytane, much reduced in proportion to pristane in control lobster meat, compared to the proportion to pristane in suspect lobster meat, or compared to the oil proportions, is also thought to have a primarily mineral origin (Meinschen 1969). "Polluted" lobster meat hydrocarbons were, in most respects, similar to control lobster meat hydrocarbons except that background "envelope" and normal hydrocarbon levels were relatively much higher (Fig. 2). Total hydrocarbons amount to 12–16 ppm of the original meat — up to 8 times higher than in control meat. The isomer ratios (Table 1) are very similar to those calculated for marine diesel oil C. Since these numbers are thought to be characteristic of specific oils (Zafiriou et al. 1972), this diesel oil is strongly indicated as a specific source of the pollution in lobster meat.

The selective degradation or elimination patterns for hydrocarbons in crustacea are unknown, but are probably parallel to those in the American oyster (*Crassostrea virginica*) (Stegeman and Teal 1973). A reduction in the C_{17}:pristane ratio would not have had time to occur in this particular instance, facilitating comparisons. "Polluted" meat hydrocarbons and diesel oil C also have scores of minor components in common present in the same relative amounts. Although the case for pollution of the lobster meat is well established on a qualitative level it is open to question on a quantitative level.

Quantities of hydrocarbons were estimated on the basis of gas-chromatographic response (relative to standards and commercial diesel oils), and are accurate within the ranges indicated. Spectroscopic quantitation methods (Mackie et al. 1972; Zitko and Carson 1970; Zitko 1970) give an accurate measure of aromatic content rather than total hydrocarbon content and were therefore not attempted.

The total hydrocarbon content of the polluted lobster meat falls well within the range of natural background or pollution background in molluscs, (Farrington and Quinn 1973), algae (Youngblood and Blumer 1973), fish (Ackman and Noble 1973), commercial oils (Ackman 1971), and other animals (Scarratt and Zitko 1972). Hydrocarbon pollution may thus be unproven in a quantitative sense, in this case. The definition of organoleptically acceptable hydrocarbon limits must obviously be made on a taste panel assessment of acceptability to the consumer. These limits could probably best be formulated on the basis of aromatic content since aromatics are the major cause of physiological and commercial concern and are readily quantitated by spectroscopic methods. Gas chromatography should be used in conjunction with spectroscopy to determine specific sources of oil in cases of pollution and to estimate nonaromatic hydrocarbon levels which might show specific geographic, seasonal, or species variations.

Acknowledgments — Information concerning the oil spill and all canned lobster meat was supplied by the Department of the Environment, Fisheries Inspection Laboratory, Halifax, N.S.

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Estimating Dry Weight of Live, Unanesthetized Fish by Photography

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HAWKES, C. L. 1975. Estimating dry weight of live unanesthetized fish by photography. J. Fish. Res. Board Can. 32: 321–324.

A photographic technique for indirectly estimating the dry weight of live fish is described with an example for fingerling coho salmon (*Oncorhynchus kisutch*). This technique does not utilize anesthetizing, blotting, or other harsh treatments associated with weighing live fish. It was developed for studies on which size, weight, etc., must frequently be obtained with a minimum of damage to the fish. The example given is the development of the regression equation correlating actual dry weights with photographically measured indices of lateral area. The equation obtained has a coefficient of determination (r^2) of 0.954.

HAWKES, C. L. 1975. Estimating dry weight of live unanesthetized fish by photography. J. Fish. Res. Board Can. 32: 321–324.

L'auteur décrit une méthode photographique permettant d'estimer indirectement le poids sec de poissons vivants. Il donne comme exemple des alevins de saumon coho (*Oncorhynchus kisutch*) de la grosseur du doigt. La méthode n'exige pas d'anesthésie, de séchage ou autres durs traitements associés à la détermination du poids sec de poissons vivants. La méthode a été mise au point en vue d'études requiérant de fréquentes mesures de taille, poids, etc., avec un minimum de dommages pour les poissons. Dans l'exemple, l'auteur développe l'équation de régression liant les poids secs réels avec des indices de surface latérale mesurés photographiquement. L'équation a un coefficient de détermination (r^2) de 0.954.

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THE usual procedures for weighing or measuring live fish cause significant stress. Harmful factors include anesthetization, desiccation, oxygen starvation, and damage to the epidermis and its mucous covering from blotting and handling. Further, the accuracy of weighing is affected by the amount of water carried on the fish's surface. These problems are greatest when small fish are used. Present methods for photographically measuring fish require anesthetics (Bushuev 1969) or the additional steps of making a photographic print and using a complicated procedure for measuring the images of fish that were in a flexed position (Martin 1967). Also, a measurement of fish depth has not been incorporated.

The photographic technique described here reduces stress by minimizing handling and eliminating the need for anesthetizing and blotting the fish. It also eliminates the inaccuracy in weighing caused by variation in amount of water carried on the surface of the fish. A high resolution photographic negative of the lateral view of the fish is obtained. Length from the anterior edge of the pupil to the posterior edge of the caudal peduncle and depth at the point of maximum depth, excluding fins, of each fish image are precisely measured. The dry weight of the fish is calculated from a correlation between dry weight and the product of fish length times depth as previously determined in calibration experiments.

Materials and methods — To obtain adequate lateral view photographs of fish, a plexiglass holding device (Fig. 1) is used to position the fish in perfect lateral view and in focus before a camera.[1] The holding device is fastened inside an aquarium and has a place to put identifying notations and calibration markings on exchangeable white plastic plates.

The aquarium side to which the device is clamped forms a 5- × 13-cm window through which the photographs are taken. The wall (Fig. 1D) of the plexiglass holding device opposite this window is moveable and is used to bring each fish sideways into the same plane of focus before the window. This wall is manipulated and kept in proper alignment by an

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[1]Detailed plans for this device are available from C.L.H.

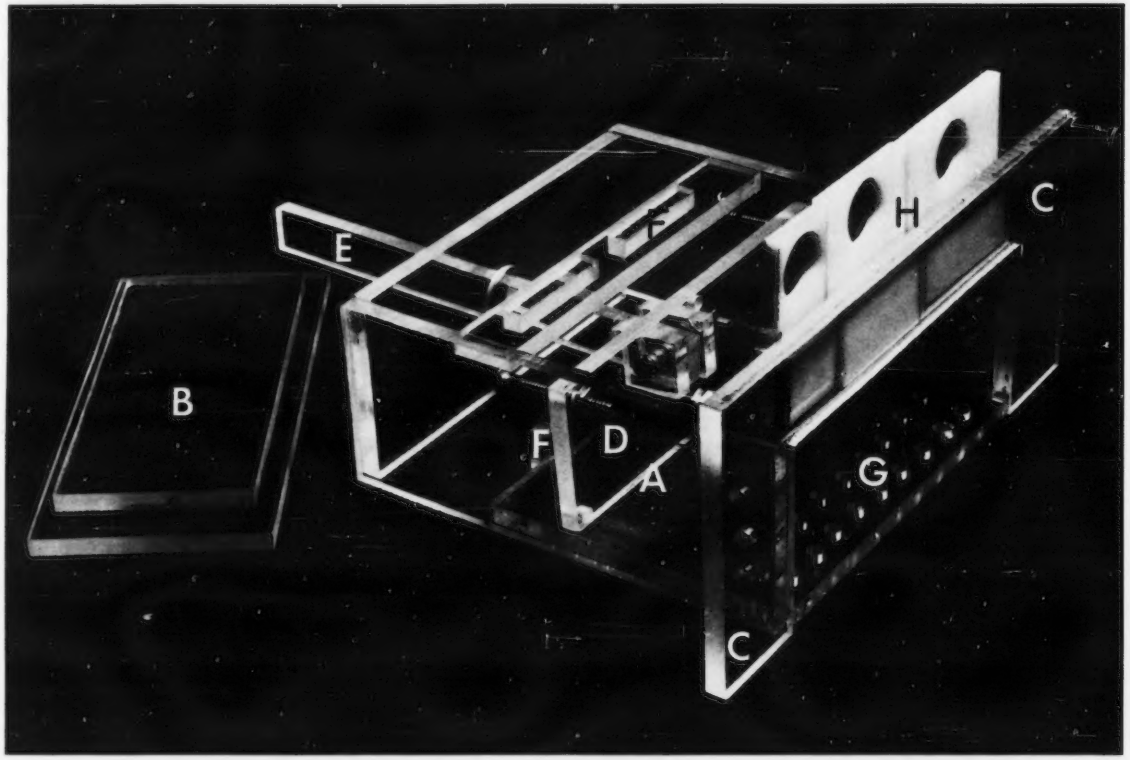

FIG. 1. Plexiglass photographic fish positioning device. A, fish chamber; B, chamber lid; C, wings for clamping chamber to the side of a glass aquarium; D, rear moveable wall of chamber used for positioning fish sideways in the plane of focus against the aquarium wall; E. handle for manipulating and keeping the moveable wall aligned; F, stops to the rearward movement of the moveable wall; G, holes to allow water to escape as the rear wall is brought forward; H, plates for writing identifying notation and for calibration markings.

Hawkes – J. Fish. Res. Board Can.

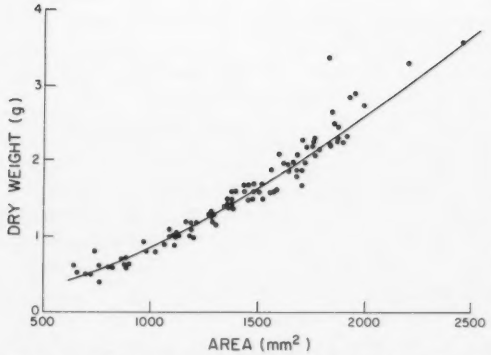

FIG. 2. Regression of fish oven-dry weight (70 C, 48 h) vs. side-view area (length × depth).

attached handle passing through a guide hole. Prior to putting a fish into the chamber (Fig. 1A) the rear moveable wall is pulled fully back. Then the fish can be inserted through the top of the chamber and contained when the lid (Fig. 1B) is put in place.

Larger or smaller versions of this device can be used as needed. However, it is best to use the smallest size possible for the fish being measured since the camera must be positioned to include the entire area of the window in the photograph. The window dimensions determine the maximum total length and depth of fish that can be measured. Maximum resolution is achieved when the fish takes up as much of this area as possible.

A 35-mm camera with a 55-mm lens is positioned 33 cm from the fish. Longer lenses (135–200 mm) are recommended to reduce variation due to differences in angle of view from small to large fish and due to differences in position.

Illumination with an electronic flash ($\frac{1}{1000}$ of a second) eliminates any degradation of the photographic image caused by movements of the fish. A single flash unit is adequate, but two units directed at the fish from each side of the camera will eliminate most undesirable shadows. Since the duration of an electronic flash is too short for one to see its reflection on the aquarium side, an incandescent bulb can be used to properly locate the flash units. Very high resolution thin emulsion films are required to provide photographic images that can be used for precise measurements of the fish images. These films must be processed in specially formulated developers (e.g. H&W Control Developer Concentrate, H&W Co., St. Johnsbury, Vt.[2] [Meyers 1970]) to reduce the extreme contrast characteristic of very high resolution films. Otherwise, it would be impossible to see the entire outline of the fish. To enhance the fish outline, a thin sheet of colored (e.g. black) plastic is attached to the moveable wall as a background. The color should be chosen for maximum contrast with the areas of the fish that will be used for delineation.

A photographic enlarger and a ruler are used to measure the length and depth of the fish image in each photographic negative. The image of the fish and calibration markings are projected as largely as possible without dimming the image too much. Precise vertical alignment of the enlarger and projection surface is very important in avoiding distortions

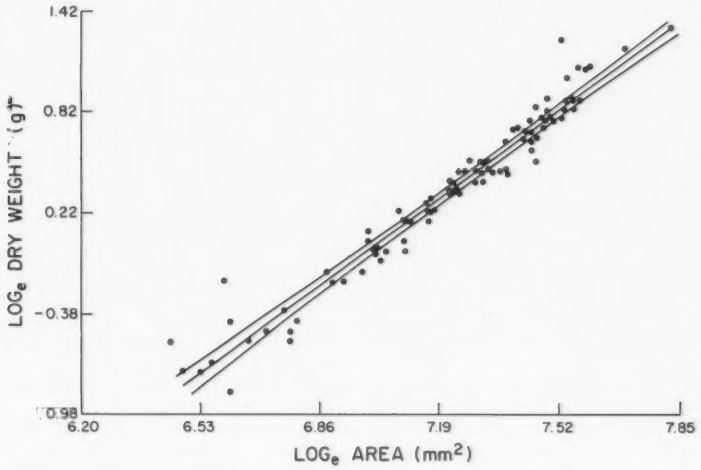

FIG. 3. Regression of log$_e$ fish dry weight vs. log$_e$ side-view area with 95% confidence limits.

[2]The use of trade or corporate names does not imply official endorsement by the U.S. Department of Agriculture to the exclusion of others.

which would give inaccurate measurements. Therefore, projection on a wall or floor rather than the enlarger base board is not advised unless alignment is precisely controlled. Measurements of the projected images of fish are converted to their actual dimensions by means of calibration markings included in the photographic negative of each fish.

Example and discussion — In a calibration experiment, 100 fingerling coho salmon were measured by the photographic procedure described, oven-dried for 48 h at 70 C, and weighed. The dry weight and indices of lateral area (the product of length and depth) were plotted (Fig. 2), and an equation was fitted to the points after transforming the values to natural logarithms. The equation obtained was $\log_e Y = -11.117 + 1.589 \log_e X$, with a coefficient of determination (r^2) of 0.954, where X is the index of lateral area in mm^2 and Y is dry weight in grams. The 95% confidence limits to the regression line were also plotted (Fig. 3).

The relationship established by this equation is used to determine the dry weight of experimental fish. Regression equations can also be constructed for the relationship between fish size (obtained photographically) and other parameters correlated with fish size. The regression of size and estimated weight can be used with fish length to calculate approximate coefficients of condition.

This technique of photographically measuring fish, constructing regression equations, and subsequently measuring experimental fish is of particular value in studying the effects of environmental stress on fish growth and condition where handling stresses must be reduced to a minimum. The technique has been used successfully in tests of chronic oral toxicity (Miller et al. 1973).

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BOOK REVIEWS/COMPTES RENDUS

The Urohyal in Fishes. *Takaya Kusaka. University of Tokyo Press. 1974. 320 p., 10 text-figures +700 figures. $50.00. Distributed by International Scholarly Book Services, P.O. Box 4347, Portland, Oreg. 97208, USA.*

Comparative studies have appeared in recent years on the branchial and hyoid arches. This book considers variation in the teleostean urohyal, a median ventral bony element lying between these arches and linking them with the shoulder girdle.

A short introduction presents the terminology and methodology used and makes generalizations about the form of the urohyal. An impressive 714 species representing 460 genera, 184 families, and 21 orders were investigated. The specimens studied were mainly from Japanese waters but these were supplemented by some material from the South Pacific and Indian oceans. Unfortunately the author did not secure specimens from eight orders and several suborders to complete the study.

The body of the text consists of illustrations and descriptions of the material studied. Line drawings depict the urohyal of each species in dorsal, lateral, ventral, front, and rear views accompanied by a 10-mm scale. The drawings are clear, and judging from a few with which I compared specimens, are accurate. The urohyal displays a remarkable variety of forms from scimitar to hook, paddle, and wing shapes. The text accompanying each drawing includes the scientific name, the locality at which the study material was collected, measurements and proportions, and, on occasion, comments comparing the urohyal with that of related species. The volume is completed by a short list of references and indices of scientific and Japanese names. The book is beautifully printed in sepia on tinted paper and is well bound.

Takaya Kusaka has contributed significantly to our knowledge of the form of the teleostean urohyal. He has made useful generalizations on the relationship of the form of the urohyal to the proportions and behavior of the fish. He forbore deducing taxonomic relationships from his data, thus providing a challenge to some enterprising taxonomist. The book will also be of value to the evolutionist and osteologist. Despite the many figures, the price of $50.00 is high and probably beyond the budget of most.

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Diseases of fish. *L. E. Mawdesley-Thomas, K. W. Burris, and J. L. Knuckles et al. Information Corp., New York, N.Y. 1974. 277 p. $17.50.*

This is not a "book" in the usual sense of the word, and its title is misleading. The hardcover "book" is a "grab bag" assemblage of photoduplicated copies of 25 generally unrelated original papers that were published elsewhere. Bibliographic data for each are given under "Credits and Acknowledgments" but not on the entries themselves.

Authors of the first three papers (the second author of one of the papers is not included) are listed on the title page; one may conclude that the "book" has 25 authors.

A section entitled OCCURRENCE begins with an 8-page review paper on fish diseases. Nine parasitology papers follow, one concerns Australian acanthocephalans, the others report on North American metazoans.

The second section is entitled PATHOLOGY AND ETIOLOGY, and it contains two papers on pathologic changes evoked by parasites and the classic quartet of papers on Ulcerative Dermal Necrosis by Roberts and his co-workers. A note on an oil-caused fish kill and a paper on salmon blood and plasma coagulation in health and disease complete the section.

MICROBIOLOGY AND VIROLOGY, consists of one paper each on microsporidians, copepods, channel catfish virus, and a nematode. MALIGNANT DISEASES consists of two illustrated notes and a paper on benign conditions. An author and key-word title index separate the papers from a 44-page listing of summaries of current research from the Smithsonian Science Information Exchange data base for November 1973.

Justification for printing this "book" is not readily apparent. It may have some value to those who do not have ready access to the literature, but it is greatly overpriced at $17.50.

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Shallow-water gammaridean Amphipoda of New England. *E. L. Bousfield. Comstock Publishing Associates, Ithaca, London. 1973. xi + 312 p., illust. $17.50.*

The book provides keys to nearly 200 species of marine coastal (<30 m) brackish, near-coast freshwater, and terrestrial amphipods from the western North Atlantic, roughly from the Gulf of Maine to New York. This area thus covers one of the centers of biological research, the Woods Hole region. Some 125 species are figured. Short introductory chapters on the general morphology and biology of the "scuds" precede the taxonomic section.

In my opinion the book will prove very valuable, and not only because of its beautiful illustrations, for New England coastal students. I must add that several of the keys are not very useful, since they contain contradictory couplets (e.g. *Gammarus duebeni* will, according to some of its characters, key out as *Gammarus*; according to others, as *Marinogammarus*).

The diagnoses and keys are valid only for the New England fauna, but this can be considered an advantage, since students unfamiliar with the group will find the simplified characterizations easier to deal with. It is unfortunate that this book of chiefly local significance has been chosen by its author as a medium to propose some far-reaching taxonomic revisions with worldwide implications. In the case of the family Corophiidae his ideas appear justified; in the case of the Gammaridae (subdivided into three families), I find it hard to follow the author's thoughts. The diagnoses are rather incongruent, e.g. for the Gammaridae s. str. it says "eyes lateral," for the Melitidae "eye rounded, small." Apart from the fact that these formulations form a poor contradistinction, in all European Melitidae that I know of, the eyes are lateral, just as in the Gammaridae; and in scores of Gammaridae, e.g. the *Gammarus simoni* complex, the eye is small and rounded.

Similar difficulties arise when one tries to locate dorsally smooth Gammaridae, as *Neogammarus*, or blind forms, as *Niphargus*, or Gammaridae with narrow marsupial plates, as *Chaetogammarus*. Maybe Bousfield's ideas are valid, but the formulations are not always convincing. I await a more detailed publication on this subject, reaching beyond the local scope. However, this piece of restraint does not affect the general utility of a fine book.

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(R18)

COMING NEXT MONTH / À PARAÎTRE LE MOIS PROCHAIN

Midsummer crustacean zooplankton communities in acid-stressed lakes **W. G. Sprules**

Two new species of *Protanypus* Kieffer, with keys to Nearctic and Palaearctic species of the genus (Diptera: Chironomidae) **O. A. Sæther**

Population dynamics of limnetic Cladocera in a beaver pond **J. K. Kwik and J. C. H. Carter**

Biodegradation of di-*n*-entyl phthalate and di-2-ethylhexyl phthalate in freshwater hydrosoil **B. T. Johnson and W. Lulves**

Tidal and diurnal effects on zooplankton sample variability in a nearshore marine environment **D. D. Sameoto**

Notes

Annual growth rates of mooneye, *Hiodon tergisus*, in the Assiniboine River **C. L. Glenn**

Use of sodium pentachlorophenate and dehydroabietic acid as reference toxicants for salmonid bioassays **J. C. Davis and R. A. W. Hoos**

Fecundity comparisons for various stocks of lake whitefish, *Coregonus clupeaformis* **M. C. Healey and C. W. Nicol**

Embryonic development, early growth, and meristic variation in rainbow trout (*Salmo gairdneri*) exposed to combinations of light intensity and temperature **W. H. Kwain**

Amino acid supplementation of casein in diets of Atlantic salmon (*Salmo salar*) fry and of soybean meal for rainbow trout (*Salmo gairdneri*) fingerlings **G. L. Rumsey and H. G. Ketola**

Occurrence of pink salmon (*Oncorhynchus gorbuscha*) in Lake Huron **J. J. Collins**

Perfusion fixation of whole fish for electron microscopy **D. E. Hinton**

Perspectives

Dynamics of exploited whitefish populations and their management with special reference to the Northwest Territories **M. C. Healey**

RECENT PUBLICATIONS / RÉCENTES PUBLICATIONS

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Les nombres qui suivent les titres de chacune des séries désignent les établissements suivants à la fin de la section

Recent Studies

Until 1970, reprints of these articles had been included in an annual volume of "Studies," composed of two parts. The final binding was the 1969 Studies, Part 2, which included No. 1355. These are no longer bound and distributed. We continue, however, to number and list all articles on investigations published in other periodicals, which henceforth may be obtained in reprint form only from the author or originating establishment.

Récentes études

Jusqu'en 1970, ces articles étaient réimprimés, puis reliés dans un volume annuel d'études comprenant deux parties. Les études de 1969, partie 2, incluant le n° 1355, ont été les dernières à être reliées. Nous ne faisons désormais plus la reliure ni la distribution. Nous continuons cependant de numeroter les articles et d'en établir des listes d'études sur des recherches mentionnées dans d'autres périodiques. On peut donc se les procurer, sous leur forme réimprimée seulement, de l'auteur ou du service dépositaire.

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2010. RUDD, J. W. M., R. D. HAMILTON, AND N. E. R. CAMPBELL. 1974. Measurement of microbial oxidation of methane in lake water. Limnol. Oceanogr. 19(3): 519–524. (4)

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Translation Series

The following translations have been prepared by or for the Fisheries and Marine Service, for use in its investigations. A limited number of copies are available for distribution as long as the supply lasts, or may be obtained by interlibrary loan, from the station where the translation has been deposited. Information on translations generally may be obtained from the Office of the Editor.

Traductions

Les traductions dont il est question ci-après ont été préparées pour ou par le Service des pèches et des sciences de la mer, à des fins de recherche. On peut en obtenir un nombre limité d'exemplaires ou se les procurer par prêts entre bibliothèques, aux stations dépositaires de ces textes. On peut en général se renseigner sur ces traductions au Bureau du rédacteur.

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The Service also publishes a series of *Bulletins*, each on a specific topic. These are for sale by Information Canada, Ottawa, and remittances must be in advance, payable in Canadian funds to the order of the Receiver General of Canada. *Bulletins* are also available from local booksellers and Information Canada bookshops throughout Canada.

The *Annual Report* of the Board and the biennial *Review* of investigations are available free, on request. All publications may be referred to at establishments located at: Ottawa, Ont.; Nanaimo, Vancouver, and West Vancouver, B.C.; Winnipeg, Man.; Ste. Anne de Bellevue, Que.; St. Andrews, N.B.; Halifax and Dartmouth, N.S.; and St. John's, Nfld.

Le Service publie également une série de *Bulletins*, chacun traitant sur un sujet spécifique. Les publications de l'ORP sont vendues par Information Canada à Ottawa. Les remises, payables d'avance en monnaie canadienne, doivent être établies à l'ordre du Receveur général du Canada. Les *Bulletins* sont aussi en vente chez votre libraire et dans les libraries d'Information Canada.

Vous pouvez obtenir le Rapport Annuel et la Revue biennale gratuitement. On peut consulter ces ouvrages aux établissements situés à Ottawa (Ont.), à Nanaïmo, à Vancouver, et à Vancouver Ouest (C.-B.), à Winnipeg (Man.), à Ste-Anne-de-Bellevue (P.Q.), à St-Andrews (N.-B.), à Halifax et à Dartmouth (N.-E.), et à St-Jean (T.-N.).

Journal of the Fisheries Research Board of Canada

Guide for Authors

A more detailed guide on preparation of manuscripts from theses is available from the Office of the Editor.

Content

We invite submission of manuscripts in the broad field of fisheries management, ocean science, and aquatic environments relevant to Canada.

Articles: Reports of research studies of broad scope that are original contributions to science.

Notes: Differ from papers on the basis of scope or completeness, rather than quality of research. They may be on problems with narrow, readily defined limits; on work that is largely confirmatory; on methodology or research techniques; on advances in knowledge arising as by-products of broader studies; on progress in projects that were discontinued; or on preliminary findings that warrant publication before problems are resolved. They may be organized with as much flexibility as articles except that they have no centered headings and should occupy no more than four printed pages.

Reviews: Detailed articles that are critical appraisals of broad areas of investigation, particularly in fields that have shown rapid development in recent years.

Perspectives: Essays of opinion or hypothesis on fisheries, ocean science, aquatic environments, or related topics that are of concern to the professional and layman. References are desirable; informal style is welcomed.

Letters: Opinion on topics recently under discussion in the *Journal*; preferred maximum length, 500 words. The Editor reserves the right to shorten letters and to make other changes to accord with the style of the *Journal*.

Editorial Practices

The *Style Manual for Biological Journals* (3rd ed. American Institute of Biological Sciences, 3900 Wisconsin Ave. NW., Washington, D.C.) is used as the authority in matters of form. The standards for spelling are: *Webster's Third New International Dictionary* and *le Grand Larousse Encyclopédique*. Titles of periodicals are abbreviated as in the *Biological Abstracts* list. References to literature are made by the name-and-year system. The *Journal* follows the names and spelling recommended in *A List of Common and Scientific Names of Fishes from the United States and Canada* (Third Edition, 1970) Special Publication No. 6 of the American Fisheries Society.

Series titles should be avoided. An *abstract* is required for all manuscripts, and should state concisely, in less than 250 words, what was done, the essentials of the new knowledge, and the main conclusions. Abstracts are published in English and French; those submitted in one language only are translated by the *Journal*.

The Editor normally submits each manuscript to at least two *referees*. Contributors are encouraged to suggest at least four names to be added to the list from which referees may be selected.

Illustrations should be submitted in triplicate as photographs (*glossy prints*) no larger than a standard page and must accompany the manuscript. Prints should not be mounted, stapled, or clipped. Symbols, letters and numerals must be neat, and at least 1.5 mm high when the illustration is reduced to the appropriate size. Drawings should, when possible, be grouped to facilitate comparisons.

Proofs are sent to the author, who is responsible for correcting only errors in typesetting. *Alterations to the content must be avoided.*

Fifty *reprints* are supplied free. Additional reprints may be purchased at the time of publication (an order form is sent out with the galley proof). All orders should be sent to the Printing Department, University of Toronto Press, 5201 Dufferin Street, Downsview, Ont. M3H 5T8. Invoices relating to the reprints will be sent out by the Printer. A charge of $30.00 per page is made for articles published. When submitting a manuscript, an author should state that his institution will accept this charge.

Checklist on Manuscripts

Attention to the following questions will expedite appraisal of manuscripts by referees and editors.

Are the findings, interpretations, and conclusions adequately documented and relevant to the purpose of the study?

Are all the tables and figures needed, and organized to facilitate comparisons? Are there inconsistencies between tables or figures and the text, or within the text? Should some of the data be made available separately, in a manuscript report or at a data repository? If you use statistical analysis are the statistics subordinate to the research?

Would any of the text be clearer if condensed? Are summary statements given at the beginning of sections and paragraphs, and are details in sections and paragraphs relevant to their topics? Does the organization of the manuscript follow logically from the statement of purpose in the introduction?

Does the *title* encompass the content of the report?

Does the *abstract* give the essentials of the new knowledge?

Is the *introduction* largely limited to the scope, purpose, and rationale of the study? Is review of the literature limited to defining the problem?

Are details of *materials and methods* limited to what scientists need in understanding the design of the study and in judging adequacy of the data?

Are generalizations on the *results* adequate? Are findings distinguished from inferences from them?

Is *discussion* limited to interpretation and significance of the findings?

Journal de l'office des recherches sur les pêcheries du Canada

Guide des Auteurs

On peut se procurer au bureau du Directeur de la publication un guide plus détaillé pour la préparation de manuscrits à partir de thèses.

Sujets

Nous désirons recevoir des manuscrits s'insérant dans le vaste domaine de la gestion des pêches, des sciences de la mer et des milieux aquatiques au Canada.

Articles: Comptes rendus d'études d'une portée générale, constituant des contributions inédites à la science.

Notes: Se distinguent des articles par la portée et le degré d'achèvement plutôt que par la qualité des recherches. Elles peuvent traiter de problèmes particuliers et bien définis; de travaux qui sont en grande partie confirmatoires; de méthodologie ou de techniques de recherche; de progrès de la connaissance découlant d'études plus vastes; de développements dans des projets abandonnés; ou de découvertes préliminaires qui méritent d'être publiées avant la solution de problèmes à l'étude. Les notes peuvent être présentées d'une façon aussi souple que les articles, mais elles ne doivent pas porter d'en-têtes centrés ni occuper plus de quatre pages.

Critiques: Articles détaillés constituant une appréciation critique de vastes champs de recherches, particulièrement dans les domaines ayant connu de rapides progrès au cours des dernières années.

Perspectives: Essais de l'opinion ou de l'hypothèses sur les pêches, les sciences de la mer, les milieux aquatiques, ou sur des sujets connexes qui intéressent professionnels et profanes. Il est préférable de donner des références et d'écrire, si possible, dans un style simple.

Lettres: Opinions sur des sujets exposés dans le *Journal*. Longueur maximale préférable: 500 mots. Le directeur se réserve le droit le raccourcir les lettres et de faire d'autres modifications dans le style du *Journal*.

Dispositions générales

Le *Style Manual for Biological Journals* (3e éd., publié par l'American Institute of Biological Sciences, 3900 avenue Wisconsin N.O., Washington, D.C.) sert de guide quant à la forme de présentation. Le *Webster's Third New International Dictionary* et le *Grand Larousse Encyclopédique* font autorité pour l'orthographe des mots. Les titres de périodiques sont abrégés suivant les *Biological Abstracts*. Les références bibliographiques dans le texte doivent suivre le système nom de l'auteur-année. Pour les noms de poissons et leur orthographe, le *Journal* suit les recommandations contenues dans *A List of Common and Scientific Names of Fishes from the United States and Canada* (Troisième édition, 1970), Publication spéciale No. 6 de l'American Fisheries Society.

Il faut éviter les *articles en série*. Un *résumé* doit accompagner chaque texte et doit exposer brièvement (moins de 250 mots) le travail accompli, les points essentiels des connaissances acquises et les principales conclusions. Les résumés paraissent en anglais et en français, le *Journal* veillant à la traduction dans le cas des présentations unilingues.

Le directeur soumet d'ordinaire chaque texte à l'approbation de deux *arbitres* ou plus. Nous encourageons les collaborateurs à suggérer au moins quatre personnes dont les noms pourraient figurer sur la liste des arbitres.

Les *illustrations* doivent être sous forme de photos (*sur papier glacé*), ne pas dépasser les dimensions d'une page normale et accompagner le texte. Elles ne doivent pas être montées, coupées ni brochées. Les symboles, lettres ou chiffres doivent se détacher avec précision et avoir au moins 1,5 mm de hauter lorsque l'illustration est réduite à la grandeur voulue. Les dessins doivent, autant que possible, être groupés pour faciliter l'étude comparative.

L'auteur doit revoir les *épreuves* qu'on lui remet et en corriger seulement les erreurs typographiques. *Il doit éviter de changer le contenu du texte.*

Cinquante *tirés à part* sont fournis gratuitement. Les tirés à part peuvent être achetés au moment de la publication. Un bon de commande à cet effet accompagne les épreuves; il doit être retourné en même temps que celles-ci, non pas au bureau de la rédaction, mais au Printing Department, University of Toronto Press, 5201 Dufferin Street, Downsview, Ont. M3H 5T8, qui se chargera de la facturer. Les frais sont de $30.00 par page pour la publication des articles. Quand un auteur soumet un manuscrit, il doit spécifier que son institution accepte les frais.

Listes de contrôles pour manuscrits

Une attention particulière aux questions suivantes facilitera l'évaluation des manuscrits par les arbitres et les éditeurs.

Les découvertes, interprétations et conclusions sont-elles bien documentées et pertinentes au but de l'étude?

Les tableaux et figures sont-ils tous nécessaires et agencés pour faciliter les comparaisons? Y a-t-il contradiction entre les tableaux ou les figures et le texte, ou dans le texte même? Est-ce que certaines données devraient être présentées séparément, dans un rapport manuscrit ou dans un dépôt de données? Si vous faites une analyse statistique, la statistique cède-t-elle le pas à la biologie?

Le texte y gagnerait-il en clarté s'il était plus condensé, en tout ou en partie? Contient-il des exposés sommaires au début de chaque section ou paragraphe, et les détails de ces sections et paragraphs son-ils pertinents au sujet traité? La marche du manuscrit est-elle conforme à la logique énoncée dans l'introduction?

Le *titre* est-il concis, tout en étant représentatif du contenu du rapport?

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L'*introduction* se limite-t-elle à une esquisse de la portée, du but et des raisons de l'étude? La revue de la littérature se limite-t-elle à la définition du problème?

La section *matériel et méthode* contient-elle le minimum de détails qui permettront aux autres scientifiques de saisir les grandes lignes de l'étude et de juger de la valeur des données?

Les généralisations sur les *résultats* sont-elles adéquates? Permettent-elles de distinguer entre constatations et déductions?

La *discussion* est-elle limitée à l'interprétation et à l'évaluation des découvertes?

Journal of the Fisheries Research Board of Canada

Guide for Typists Preparing Copy

Read "Guide to Authors" in the current volume of the *Journal*. When in doubt about how to type or arrange details, follow *Journal* practice.

Type the manuscript on white bond paper, not flimsy, preferably size 21.5 × 28 cm (8.5 × 11 inches). If possible, use paper with the lines numbered in the margin. Leave all margins at least 2.5 cm wide.

Double-space all parts of the manuscript, including tables, captions for illustrations, footnotes, and the reference list. Underline only material that is to be printed in italics. Indent the first line of each paragraph in the text and of each footnote.

In titles and headings, capitalize only the first word and proper names and adjectives. For small capitals, use small letters.

Indicate all text footnotes by superscript arabic numerals. Number them consecutively throughout the manuscript, starting with those on the title page. Place each footnote immediately below the reference to it, separating it from the text by horizontal lines.

On the first page type only the title, the authors' names and affiliations, related footnotes, and the running head (see Appendix A). Type the abstract on a separate page (see Appendix B). Number all pages, beginning with the title page and including those for tables and captions for illustrations.

Have the author check the finished manuscript, and send the ribbon copy and two other clear copies to the Office of the Editor.

Tables: Examine the current volume of the *Journal* to see how tables are set up (see Appendix C). Note especially the following concerning *numbered tables:*

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Use horizontal lines above and below the headings and below the columns, and seldom elsewhere. Never use vertical lines; leave extra spaces instead.

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Show all symbols clearly. In the margin, spell out Greek letters the first time they occur, and distinguish between the small letter *l* and the numeral *one*, and between the capital letter *O* and the numeral *zero*. Make other explanatory notes similarly.

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Spellings: About 100 words common in fisheries journals are listed in Appendix F.

Appendices: Appendices A to F are available on request from the Office of the Editor.

Journal de l'office des recherches sur les pêcheries du Canada

Guide pour les secrétaires d'auteurs

Lisez le "Guide des Auteurs" reproduit dans l'édition courante du *Journal*. Si vous avez des doutes sur la façon de dactylographier le texte ou d'en agencer les détails, suivez la pratique du *Journal*.

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Tout le manuscrit, y compris tableaux, légendes de figures, renvois et références bibliographiques, devra être dactylographié à double interligne. Ne soulignez que les mots qui devront être imprimés en italique. Mettez en retrait la première ligne de chaque paragraphe du texte et de chaque renvoi.

Dans les titres et les en-têtes, n'employez la majuscule que pour le premier mot et les noms propres. Pour les petites majuscules, employez les miniscules du dactylographe.

Indiquez tous les renvois du texte par des chiffres arabes suscrits. Numérotez les renvois consécutivement par tout le texte, à commencer par ceux de la page titre. Placez chaque renvoi directement sous sa référence, le séparant du texte par des lignes horizontales.

La première page ne doit contenir que le titre de la publication, le nom et l'affiliation de ou des auteurs, les renvois appropriés et le titre courant (voir Appendice A). Dactylographiez le résumé sur une page séparée (voir Appendice B). Numérotez chaque page, à commencer par la page titre, et y compris celles qui contiennent les tableaux et la légende des figures.

Voyez à ce que l'auteur vérifie le manuscrit final, et soumettez la première copie et ainsi que deux copies lisibles au bureau du Directeur de la publication.

Tableaux. Consultez une copie du *Journal* pour la composition des tableaux (voir Appendice C).

Portez une attention particulière à ce qui suit au sujet des *tableaux numérotés*.

Dactylographiez chaque tableau sur feuille séparée, vous servant de papier sans lignes numérotées.

Numérotez les tableaux en chiffres arabes.

Tracez des lignes horizontales au-dessus et au-dessous des en-têtes et au-dessous des colonnes, rarement ailleurs; ne tracez jamais de lignes verticales; laissez plutôt des espaces supplémentaires.

Indiquez les renvois dans les tableaux par les minuscules suscrites et placez les renvois au bas du tableau.

Placez les tableaux à la suite des références bibliographiques.

A l'endroit où le tableau est mentionné pour la première fois, indiquez dans la marge "Tableau — près d'ici."

Remarquez que les *tableaux dans le texte* ne sont pas numérotés, sont dactylographiés avec le texte et ne requièrent que rarement des lignes horizontales (voir Appendice C).

Figures: Envoyez avec le manuscrit trois photos *sur papier glacé* (21,5 × 28 cm ou moins) pour chaque illustration. Dactylographiez le nom de l'auteur et le numéro de la figure sur une lisière de papier que vous collerez au dos. N'écrivez pas à la main ou à la machine sur la photographie même. En plus, dactylographiez les légendes, dans l'ordre, sur feuille séparée, intitulée "Légendes des figures." Intercalez cette feuille à la suite du texte.

A l'endroit où chaque figure est mentionnée pour la première fois, inscrivez en marge "Fig. — près d'ici."

Equations, formules et expressions mathématiques: Laissez trois espaces au-dessus et au-dessous des lignes renfermant des chiffres ou des lettres suscrits et souscrits, ou encore au-dessus et au-dessous d'équations et de formules. Placez chaque composante des différentes expressions dans la position qui lui est propre (voir Appendice D).

Indiquez clairement tous les symboles. Epelez en marge les lettres grecques, la première fois qu'elles apparaissent. Faites une distinction entre la lettre *l* et le chiffre *un*, et entre la majuscule *O* et le chiffre *zéro*. Insérez de la même façon toute autre note explicative.

Références: Vérifiez le texte afin de vous assurer que toutes les références apparaissant dans la liste bibliographique sont bien mentionnées dans le texte. Dactylographiez à double interligne dans le style en usage dans le *Journal* (voir Appendice E). Les noms de revues sont abrégés suivants les *Biological Abstracts*; en cas de doute, écrivez le nom au long.

Orthographe: Une liste d'environ 100 mots communément employés dans les périodiques sur les pêches est donnée en Appendice F.

Appendices: On peut se procurer une copie des Appendices A à F sur demande au bureau du Directeur de la publication.

BULLETIN 186

The Capelin (*Mallotus villosus*) Biology, Distribution, Exploitation, Utilization, and Composition

P. M. JANGAARD

The rapidly increasing interest in the use of capelin for human consumption has led to the production of this bulletin. The following topics are examined: life history, including systematic position, common names, general description, distinctive features, meristic characteristics, growth, and feeding; occurrence; fishery procedures, handling, unloading, and use of preservatives; utilization, processing, and equipment; and chemical and nutritional composition of whole capelin, capelin meal, and oil.

The capelin, *Mallotus vilosus*, a small, pelagic fish belonging to the family Osmeridae, occurs in large quantities in the cold waters of the northern hemisphere. Distribution is circumpolar with large stocks found in the Barents Sea, around Iceland and Greenland, and in Canadian waters from Nova Scotia to Labrador. Small stocks occur on both sides of the North Pacific.

Formerly, small quantities of capelin were used for food in fresh, salted, and dried forms. At present, most capelin catches are used for production of meal and oil. However, various frozen and canned products have recently been developed and test-marketed, Japan being the leading importer and consumer.

L'intérêt sans cesse grandissant que l'on porte au capelan comme source de nourriture humaine est à l'origine de ce Bulletin. On y examine les points suivants: cycle biologique, y compris position systématique, noms vulgaires, diagnose générale, caractéres distinctifs, y compris caractéres numériques, croissance et alimentation; répartition; méthodes de capture, de manipulation, de déchargement, et usage de préservatifs; utilisation, transformation et équipement; composition chimique et valeur nutritive du capelan entier, de la farine et de l'huile de capelan.

Le capelan, *Mallotus villosus*, un petit poisson pélagique de la famille des Osmeridae, se trouve en grande abondance dans les eaux froides de l'hémisphère nord. Sa distribution est circumpolaire, et il existe des stocks considérables dans la mer de Barentz, autour de l'Islande et du Groenland et dans les eaux canadiennes, depuis la Nouvelle-Écosse jusqu'au Labrador. De petits stocks se trouvent sur les deux côtés du Pacifique Nord.

Dans le passé, de petites quantités de capelan étaient utilisées comme nourriture, à l'état frais, salé et fumé. Maintenant, la plus grande partie des prises est transformée en farine et en huile. Cependant, on a récemment développé, aprés essais de commercialisation, divers produits congelés et en conserve hermétique, le Japon étant le principal importateur et consommateur.

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